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CHROMOSOME STUDIES ON HUMAN SUBJECTS EXPOSED TO ARSENIC IN DRINKING WATER

By

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Contract # 68-03-3170

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16. ABSTRACT

A two-year study was carried out on human subjects of various ages and and backgrounds who have been drinking water containing 0.05 mg/l (0.05 ppm) or more arsenic for a period of at least 5 years. A control group that had been exposed to arsenic in drinking water at concentrations less than 0.05 mg/l was used for some aberrations and sister chromatid exchanges in the lymphocytes of the two populations with the amount of arsenic in the water they have been drinking. Besides, a questionnaire was designed to explore the incidence of skin cancer, fetal wastage, any genetic development abnormalities in the family and related parameters. Several other variables, e.g., coffee, wine and cigarette consumption, sex, residence (rural vs. urban) and exposure to chemicals, smelter and pesticides were taken into consideration. The study involved the city of and areas in the vicinity of Reno, Nevada, USA.

This study on chromosome aberrations (104 exposed and 86 control individuals) and on sister chromatid exchanges (98 exposed and 83 control individuals) did not show that arsenic at a mean level of 0.109 mg/l has any effect on these parameters. Similarly, no effect of arsenic at these modest concentrations was found on other health parameters studied.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and nonionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This was a cross sectional epidemiologic study designed to test the relationship(s) of exposure to waterborne arsenic and incidence of chromosome aberrations and sister chromatid exch nges in humans. Exposure was defined as consumption of drinking water containing at least 0.05 mg/l for at least 5 years. No statistically significant ($p<0.05$) relationships were found between the cytogenetic measurements and exposure to waterborne arsenic.

F. Gordon Hueter, Ph.D
Director
Health Effects Research Laboratory

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ABSTRACT

A two-year study was carried out on human subjects of various ages and and backgrounds who have been drinking water containing 0.05 mg/l (0.05 ppm) or more arsenic for a period of at least 5 years. A control group that had been exposed to arsenic in drinking water at concentrations less than 0.05 mg/l was used for some aberrations and sister chromatid exchanges in the lymphocytes of the two populations with the amount of arsenic in the water they have been drinking. Besides, a questionnaire was designed to explore the incidence of skin cancer, fetal wastage, any genetic development abnormalities in the family and related parameters. Several other variables, e.g., coffee, wine and cigarette consumption, sex, residence (rural vs. urban) and exposure to chemicals, smelter and pesticides were taken into consideration. The study involved the city of and areas in the vicinity of Reno, Nevada, USA.

Our data on chromosome aberrations (104 exposed and 86 control individuals) and on sister chromatid exchanges (98 exposed and 83 control individuals) did not show that arsenic at a mean level of 0.109 mg/l has any effect on these parameters. Similarly, no effect of arsenic at these modest concentrations was found on other health parameters studied.

INTRODUCTION

The metalloid arsenic (AS) is characterized by its presence in all environmental substrates. It is primarily found as As^{3+} or As^{5+}, in both inorganic and organic complexes (arsenicals) with varying degrees of toxicity, biological activity and stability. In trivalent forms it is more toxic than in pentavalent form. It is more readily absorbed by animals in organic than in inorganic combinations. Human body contains 40-3000 ppb arsenic (Jukes, 1979), primarily obtained from various foods and drinks, notably sea food and wines. Sea weeds also contain high quantities of the metalloid.

The metalloid has gained notoriety primarily as a suspect carcinogen in drinking water and evidence has been forthcoming showing several types of ill effects including death (Zaldivar, 1972, Lisella et al., 1972, Tseng et al., 1968). Generally, chronic exposures to elevated levels of As produce weakness, muscular aching, prostration, cutaneous manifestations, localized pigmentation, hyper-keratosis especially over hands and soles, polyneuritis (including numbness, tingling, prickly sensation) and a decrease in sensitivity to touch, pain and temperature (Vallee et al., 1960; Heymen et al., 1956). More severe intoxication may also result in kidney degeneration, liver cirrhosis, gastrointestinal catarrh and bone marrow injury. Anemia resulting from consumption of contaminated soy sauce and milk (Mizuta et al., 1956) and electromyographic abnormalities among the residents in the vicinity of arsenic smelting operations (Tsuchiya, 1977) have also been reported. Borgono and Grieber (1972) and Zaldivar (1974) even reported deaths due to cardiovascular problems, especially thrombosis of mesentric and brain arteries, myocardial infarction and narrowing of coronary arteries.

The main concern of USEPA, however, has been the carcinogenic potential of As when ingested in various forms, especially in drinking water. A few scattered reports from abroad have linked the presence of As in drinking water to cancer of skin. Tseng et al. (1968), for example, reported an episode of water-borne As poisoning in southwest Taiwan. Skin cancer and keratosis were reported by these workers in the population which had been drinking well water containing more than 0.3 mg/l of As for as long as 45 years. The prevalence of skin cancer was reported to be in direct proportion to the arsenic content of the water. More recent investigations, however, indicate that skin cancer may be due to other causative agents.

The second consideration has been a study carried out in Chile. Borgono et al. (1977) related 0.8 ppm As in drinking water supply of the city of Antofagasta during 1958-1970, with hyper-pigmentation of skin. They found a startling rate of clinical manifestations (e.g., as high as 43.7% for hyperkeratosis among individuals with abnormal skin pigmentation). In a 1976 study the dermatologic lesions were not found in children under 6 years of age because "a water treatment plant, that started to operate in 1970, was built to solve the Problem." These conclusions can not, however, be readily

accepted because of several pitfalls in the study: for example, the methodologies are not clearly explained, nor are there any specific references to the study carried out in 1971.

Other studies have also found an association of cancer with exposure to arsenic. Neubauer (1947) noted increased incidence of skin cancer in residents of certain parts of England exposed to arsenic in drinking water, and Lee and Fraumeni (1969) observed a three-fold excess of respiratory cancer deaths among smelter workers. Similarly, a study by Dow Chemical Co. showed a threefold increase in lung cancer among employees in a dry arsenical manufacturing plant over the rate for nonexposed employees (Ott et al., 1975). Of all these studies however, only the Neubauer's study considers natural exposure to As in drinking water. The concentration of As in the water was estimated at a level of 12 mg/l. Some studies have failed to demonstrate increased incidence of cancer in industrial workers despite continued exposure to high concentrations of arsenic trioxide (Snegireff and Lombard, 1951; Pinto and Bennett, 1963).

Considering the data like those available in the above reports, the USEPA thought it prudent to set some sort of national standards for maximum concentration of various types of arsenicals in drinking water in this country. The "Interim Drinking Water Regulations" allow a maximum of 0.05 mg of total As (in all forms) per liter of drinking water (EPA, 1976). The report, however, does recognize the variations in the nature of chemical combinations in which As is available and the wide range of concentration of As in various geographic areas of this vast country. The report does not, nor was it expected to, recognize that the individuals in which cancer was observed might be some susceptible genetic isolate in which As is a precipitation cofactor rather than the cause of carcinogenicity.

Some studies have been carried out in the USA of populations exposed to arsenic in drinking water. Morton et al. (1976) could not find any association between arsenic levels (approximately 0.1mgAs/l) and incidence of cancer in Oregon. Similarly, a California study failed to show evidence of any specific illness associated with As (0.1 mg/l and 1.0 mg/l) in drinking water (Goldsmith et al., 1972). To these reports may be added a recent study by Southwick et al (1980) carried out in Utah in which no evidence of As induced dermatological problems was found in individuals exposed to As between 0.18 and 0.21 mg/l in drinking water. None-the-less such studies usually show accumulation, and hence increased levels, of As in some parts or fluids of the body, e.g. hair, nails, urine, when concentrations in drinking water exceed 0.05 mg/l. Such "storage - disposal" mechanisms may represent safety systems of the human body.

Even though arsenic poisoning is known to occur at doses as low as a few milligrams per day injested for about two weeks, the effects of a total lack of arsenic in the human body is not known. Arsenic has recently been shown to be an essential element for pigs, goats (Anke et al., 1976) and rats (Nielsen et. al., 1975). Arsenic deficiency appears to cause loss of weight and even death (Davies, 1981). In chicks arsenic deficiency symptoms include increased hematocrits, decreased plasma uric acid and weak and twisted legs (Uthus and Nielsen, 1980). It has been suggested that arsenic may be necessary for utilization of zinc (Uthus and Nielsen, 1980). It may be a substitute for

phosphorus in the phosphorylation of nucleotides (see Nordberg and Anderson, 1981) although convincing evidence is still lacking.

Only limited informatio is available on the effects of arsenic on structure and function of genetic material (see Fishbein, 1981). To the best of our knowledge there are no studies on the genetic effects of naturally occurring arsenicals in drinking water to which man is exposed. A few studies on DNA, RNA and protein synthesis mainly confine to test tube experiments under artificially controlled conditions. Those dealing with effect on chromatin and chromosomes use lymphocytes of subjects exposed thereapeutically to high concentrations of arsenic.

Arsenic blocks the cells in S- and G_2- phase (Peters et al 1977). Sibatani (1959) and Peters et al (1977) found a dose dependent inhibition in incorporation of radioactively labelled nucleotides in DNA of human lymphocytes in cultures exposed to 0.1 µg or more sodium arsenate/ml. Similar inhibition, though to a lesser degree, was seen for RNA synthesis. Rather strong inhibition of DNA synthesis and protein synthesis was reported by Nakamura and Sayato (1981). Inhibition of both single strand DNA breaks and post-replication repair have been reported in Escherichia Coli (Fong et al., 1980). None-the-less evidence for true mutagenic activity of arsenic is lacking (see Nordberg and Andersen, 1981). Thus whereas DNA repair inhibition may point to carcinogenic ability of arsenic, the lack of induction of point mutations may not be entirely compatible with this property (Also see Leonard and Lauwwrys, 1980, for review).

There is ample evidence of chromosome aberrations in psoriatic patients treated with arsenic (Petres et al., 1977, 1981; Nordenson et al., 1979) and wine growers exposed to arsenic containing pesticides (Petres et al., 1977, 1981). Additionally, Nakamuro and Sayoto (1981) have shown a significantly higher frequency of chromosome abberations in cultured human skin fibroblasts and leukocytes exposed to trivalent arsenics ($NaAsO_3$, $AsCl_3$, As_2O_3) than in those exposed to pentavalent forms ($NaHAsO_4$, H_3AsO_4 and As_2O_5). These studies, along with those on colony forming ability of cells after exposure to arsenic, are compatible with others showing higher toxicity of trivalent forms than pentavalent. There is also evidence that the frequency of sister chromatid exchanges (SCE's) in psoriatic patients treated with arsenic compounds is elevated (Burgdorf et al., 1977). None-the-less when concentrations of arsenic trioxide were moderated to 12 mg As/kg body weight in Swiss albino male mice, there was no evidence of increase in the frequency of chromatid/chromosome aberrations in either bone marrow cells or spermatogenia (Poma et al., 1981).

For almost a decade, geneticists have been looking at correlation between mutagenicity and carcinogenicity of many chemicals. Bacterial studies initiated in Bruce Ames' lab at Berkeley have amply justified the slogan 'Carcinogens are mutagens'. More recently, cultured mammalian cells have been added to the battery of test organisms. These have provided a correlation between carcinogenic potentials of various chemicals and their ability to induce chromosome aberrations and SCE's, especially the latter, since a magnification in the frequency of SCE's can be observed at low concentrations of chemicals which are not effective in inducing traditional chromosome aberrations (Perry and Evens, 1975).

Therefore, if arsenic is a true carcinogen at low concentration as found in drinking water, it may effect the frequency of chromosome aberrations or, at least, SCE's in the lymphocytes of people exposed to this chemical in drinking water. It was therefore decided to study the frequency of chromosome aberrations and SCE's in the lymphocytes of a sample population exposed to arsenic in drinking water and to compare the data with those obtained from the control, 'non exposed', population sample.

SURVEY INFORMATION AND SAMPLING PROCEDURE

Like many parts of the nation, Nevada has several pockets of population which depend upon underground water either supplied through a municipal water system in large towns or through individual family wells. The town of Fallon, Nev. (Fig. 1), located approximately 55 miles northeast of Reno, Nev, has arsenic present at concentration of 0.1 mg/l in its municipal water supply. The town has a population of about 10,000 permanent residents, almost exclusively of European descent. A few isolated homes have their own wells where arsenic ranges between 0.08 mg/l to as high as 2.0 mg/l. In view of a stable, farming population and its reasonable vicinity to our lab in Reno, it was decided to study a sample of this population as the group "exposed" to arsenic in drinking water. A few individuals were also contacted in a nearby suberb of Reno, viz., Hidden Valley, where arsenic content of the well water supply is higher than the permissible 0.05 mg/l. The control group was entirely from Reno area which is served by the river water (Truckee River fed by over-flow from Lake Tahoe) with arsenic content in trace quantities not exceeding 0.005 mg/l.

A questionnaire (Appendix) was designed to obtain information about the following: sex, ethnic origin, age, occupation, environmental exposures to pesticides and other chemicals, water quality, food and drinking habits, smoking, health status, health related complaints, pregnancy complications, birth defects among the offsprings (a copy of the questionnaire is enclosed as Appendix to this report). Participation in our study was entirely voluntary; our only requirement being that the volunteer must have been drinking water containing (1) 0.05 mg/l or more arsenic for at least 5 years to be classified as "exposed" or (2) only traces of arsenic ($<$0.005 mg/l) to qualify as control. The sample was selected without meeting the individuals personally, i.e., by random phone calls from the telephone directory of the city of Fallon. The controls were primarily from the student population, the faculty and colleagues working at the University of Nevada and at Nevada Mental Health Institute in Reno or were visitiors to these places selected randomly, besides a few residents of the city. Only one call was made per individual. He/she was explained the purpose of the study, the risks involved, and the time required to answer the questionaire. If an individual accepted our request the first time, he/she was included in the study. If the individual declined, no effort was made to persuade and he/she was not contacted again. We did not ask for the reason for accepting or declining our request. It is estimated that about 1/3 of those contacted refused to participate.

Those who accepted to participate were contacted in person at their residence at a time convenient to both parties. First, the questionaire was discussed and answers filled in, preferrably by the volunteer. Then a blood sample (approximately 5 ml) was drawn by venipunture. Lastly, a sample of water from their kitchen tap was obtained in clean, sterile, plastic bottles. The appropriate consent forms were also completed, as necessary.

The average number of participants was between 2 and 3 per week. This was scheduled to avoid back-log of work and to distribute the sampling over a period of 1 1/2 years (October 1978 - May 1980). The procedure was to continue till a total of at least 150 subjects in both groups were studied. Actually a total of 191 individuals (105 exposed and 86 control) were analyzed.

LABORATORY METHODS

The telephone calls, interviews and sampling were accomplished by one of the technicians (M.F.). Once he brought the material back to the laboratory, the questionnaire and the samples were assigned a code number, the questionnaire was filed and after that we had to deal with coded samples only. The water sample was delivered to the State of Nevada Health Laboratory in Reno. This lab analyzed total arsenic content. Two methods were employed: atomic absorption using hydride generation and the colorometric method using silver - diethyldithiocarbamate. The blood sample was divided into 5, 15 ml tubes each containing 7 ml of GIBCO 1 A chromosome medium and 0.3 ml of blood. One of these tubes was harvested at 48 hour post-culture and another 72 hour post culture. This was done to achieve 1st metaphase cells for chromosome aberration studies, in as many cases as possible. In the event of the failure of 48 hour culture the back-up 72 hour culture was used. The other 3 tubes were treated with 10^{-5} to 5×10^{-5} M BrdU starting at 36 hour post-culture and harvested 36 or 48 hours later. This was done to study the SCE's. Usually the 5th tube was never needed, and generally we were successful in obtaining desirable samples of cells for aberration study and SCE's only from a single tube each.

Chromosome preparations were made by routine techniques used in our lab. The cells were pretreated for 1 hour with 0.01 µg/ml of colcemid, given hypotonic treatment with 0.075 M KCl for 10 minutes and fixed in cold 3:1 acetic acid:ethenol. The slides were coded only with numbers. For study of SCE's, the Hoescht 33258 pretreatment was followed by exposure to white light for 8 hours and then 1 hour incubation in 2 X SSC following the procedure by Perry and Wolff (1974). All slides were stained in 3% Giemsa for 5 minutes. At this point the slides were examined by the first technician (M.F.) for general quality. The principal investigator also studied samples from time to time.

The slides so prepared were handed over to another technician (M.C.). He was instructed to study up to 200 cells for chromosome aberrations, preferrably from 48 hour preparations. The classification for aberrations was standard: gaps, breaks and exchanges of chromatid or chromosome type. For study of SCE's, up to 25 cells were to be analyzed. This second technician did not have any idea whether the particular case he studied came from the exposed or control population. He entered his data under the given code number and consulted the principal investigator at the time he had any doubts. Both technicians met with the P.I. regularly once every week to report the progress.

Quality Assurance: The reproducibility of the results and uniformity of standards applied throughout the study was assured from the very beginning. The water of the City of Fallon and Reno is routinely checked for arsenic content at least once a month by the State Health Laboratory. The samples obtained from individual homes therefore turned out to serve as duplicates. A few times the timings of obtaining these samples coincided with the timing of analyses for the bulk source serving these cities. In every case the replicate samples gave identical results. Besides at one point the project director took three samples, at random, from the homes of our study subjects and had them analysed elsewhere. The results again agreed with the analysis carried out in Reno. Also, two water samples sent to us from Cincinnati were analyzed in Reno with similar interpretation by both laboratories.

For study of cytogenetic parameters, three randomly selected samples were processed in duplicate. Each replicate was analyzed separately. The results were similar both for chromosome aberrations and SCE's. Also the technician with main responsibility for analysis of slides for chromosome aberrations and SCE's had studied several thousand cells for these parameters before we started working on this project. He was therefore well versed with various types of aberrations. Besides the P.I.'s major field of research is the study of cytogenetic end points in environmental mutagenesis studies and he has published extensively on induced chromosome aberrations. The P.I., on several occassions checked random samples of cells under the microscope and compared his interpretation with that recorded by the technician. The interaction between these individuals and other members in the lab assured that no guess work is being implied at any stage of the study.

The general quality of cells was also good. At one point, the Project's Director and Dr. Peter Bercz were also shown several cells under the microscope taken from different cases at random. Examples of two cells are produced in Figure 2.

THE POPULATION SAMPLE

Detailed information on the raw data on various aspects of these studies are provided in Table I.

A total of 211 individuals were included in the study. Of the cultures set up from these individuals, 196 were successful and yielded data on chromosome aberrations, sister chromatid exchanges, or both. Two cases representing residents of Cincinnati were eliminated from further consideration, so that comparisons could be made of samples from urban and rural Nevada. Three individuals were tested twice, to check repeatability of results over a short time interval. One set of results for each of these individuals was chosen at random for inclusion in the final analysis. This leaves a total of 191 cases on which further analysis was conducted. Of these, 190 had data for chromosome aberrations and 181 had data for SCE's. One individual who was drinking well water with 2.275 ppm arsenic, 6 times greater than the second highest value, was excluded from regression analyses using arsenic as an independent variable so as not to bias these results. It should be pointed out that this individual, a 45-year-old female Fallon resident, had fewer chromosome aberrations than 70% of the entire sample and fewer SCE's than 17% of the entire sample.

Table II shows the data on distributions of individuals in the exposed and control groups with respect to seven categorical variables measured in the study. There were no significant differences between exposed and control groups with respect to sex, exposure to pesticides, smelters, chemicals, or smoking (Chi-square analysis of 2x2 contingency tables). There were more wine drinkers among control than exposed individuals ($\chi^2 = 4.66$ on 1 df, $p = 0.03$) and more exposed individuals in the rural than in the urban sample ($\chi^2 = 74.56$ on 1 df, $p < 0.001$). The latter result, of course, was anticipated as part of the design of the study. Figures 3 - 5 show the distributions of control and exposed individuals with respect to age (Fig. 3), water consumption (Fig. 4), and coffee (Fig. 5) consumption. The mean age of the control group (29.6 years) was significantly ($p < 0.001$) less than that of the exposed group (42.2 years). Mean daily water consumption was not significantly different (4.35 glasses and 5.12 glasses, respectively, $p = 0.10$) nor was mean daily coffee consumption (3.30 cups and 4.09 cups, respectively; $p = 0.13$)

DATA ON GENETIC PARAMETERS

The details of the entire raw data relevent to the study of chromosome aberrations and SCE's are provided in Table I. A histogram showing distribution of water-borne arsenic levels is produced in Figure 6. Thus, the range of arsenic content in drinking water in the control population was .005 mg/l to as high as 0.045 mg/l, and that in the exposed population was from 0.05 mg/l to 2.275 mg/l. The mean arsenic content in the water of the control group was 0.012 ± 0.001 mg/l whereas in the water of "exposed" population, not including the extreme value of 2.275 mg/l, was 0.109 ± 0.005 mg/l -- a value showing approximately 9-fold increase over that of the control. In other words the average arsenic content in the drinking water of the exposed population was 218% of the highest limit mandated by the USEPA regulations. The data provide a reasonable difference in arsenic content of the two populations upon which to base comparisons sought in this study. Additionally, the number of individuals studied in the exposed (105) and control (86) samples are reasonably good parameters to judge the effect of modest quantity of arsenic in drinking water. With these sample sizes and the observed variances, differences between control and exposed groups of approximately 0.007 chromosome aberration per cell and approximately 0.521 SCE's per cell should be detectable with 95% probability.

It is common knowledge that the frequency of chromosome aberrations and SCE's is influenced by a host of chemicals, physical agents and biological parameters. Some chemicals like several anticancer antibiotics and pesticides, physical agents like radiations and hyperthermia, biological parameters like certain genetic constitutions and ageing result in increased frequency of chromosome aberrations and SCE's. The effect of other factors e.g. alcohol consumption and cigarette smoking are equivocal. On the other extreme, foods containing caffeine are strong clastogens in the in vitro systems but no influence on chromosome breakage is demonstrable in the whole animal.

In consideration of these factors, as mentioned, the questionaire was designed to accomodate information on many of these aspects. Hence, besides arsenic content in drinking water, the other parameters recorded in table I are sex, age, exposure to pesticides, smelter, chemicals containing arsenic, quantity of water, coffee, wine and cigarettes consumed, as well as health status. These provide a survey of the relevant life style of the subject and may influence the genetic damage observed in the lymphocyte cultures. A summary of the data is provided in Table II.

The number of cells analyzed for chromosome aberrations in each case is also given in Table 1. This shows that in most cases the number of available metaphases was sufficient to study 200 cells. In 16/190 cases of successful chromosome preparations, however, the number of cells studied was less than 200 per case; amongst these only 2 cases provided us with less than 100 cells;

1 with 90 and the other with 79 cells. None-the-less, even these samples are generally considered large enough to estimate the frequency of aberrations. Hence, all these data were utilized in the final analysis of aberrations. In the case of SCE's (181 cases) the data were obtained from as many as 50 cells per individual up to case no. 71 and from analyses of 25 cells thereafter. Most laboratories around the world consider 20 cells to be optimal sample for this analysis in view of the uniformity of SCE frequency in the cell population (Perry and Evans, 1974).

The overall frequency of chromosome aberrations in our entire population ranged between 0.00 to 0.13 aberrations/cell. In our experience, the average for a healthy population lies usually between 0.03 and 0.05 aberrations per cell. Thus the range shows that whereas some individuals in the population studied for this project displayed exceptional stability for chromosome structure, the others clearly expressed a rather high frequency of aberrations. The range for SCE's in population was between 5.48 to 15.28 per cell. The upper value here, again, is higher compared to the usual range of between 7 and 10 SCE's per cell for normal, healthy individuals whose lymphocytes are treated with 10^{-5}M BrdU. Thus in case of both, chromosome aberrations and SCE's, in certain individuals some factors are operative which are responsible for an increase in the frequency of the types of genetic damage under study.

Considering all the factors discussed above in demography of the population (Table II) and the raw data on the frequency of genetic damage observed in the sample Table I, it appears that the age factor in the exposed and control population favors the odds for higher aberration frequency in the exposed group.

STATISTICAL METHODS

Because of unavoidable correlations in the sample between exposure to arsenic in drinking water and other factors which may influence frequencies of chromosome aberrations and SCE's (e.g., age, wine consumption), the data were analyzed by multiple linear regression to determine if increased amounts of arsenic in drinking water contribute to increased frequencies of chromosome aberrations and SCE's once other potentially confounding variables (see Table III for codes used below) are accounted for. This analysis proceeded as follows:

(1) The categorical variables, wine consumption, cigarette consumption, and city, were converted to dichotomus variables -- WINEGRP (0 = no wine consumed, 1 = 1 or more glasses per day), CGSGRP (0 = non-smoker, 1 = smoker), and CITYGRP (0 = rural Nevada, 1 = urban Nevada). These plus four other dichotomous variables, SEX, PEST, SMELT, and CHEM, were used as independent variables in the analysis.

(2) Since the major concern of the study was the effect of arsenic on chromosomes, three ways of representing arsenic exposure were used in the regression analysis. These were: (a) the raw data on arsenic content of the water being drunk by an individual (ARSENIC as an independent variable), (b) arsenic exposure as a dichotomous variable according to USEPA standards (less than 0.05 ppm = control, greater than or equal to 0.05 = exposed (ARSGRP as an independent variable), and (c) arsenic content of water times glasses of water drunk per day (ARSWAT as an independent variable). Separate regressions were run using each of these variables.

(3) AGE was the ninth independent variable in the analysis.

(4) Coffee consumption was skewed to the right. Therefore a logarithmic transformation (LOGCOFF = Log_{10} (COFFEE + 1) was computed, and one set of regressions used COFFEE as the tenth independent variable while another set used LOGCOFF.

(5) Three dependent variables were used in the sets of regressions which were run with the independent variables described above. Examination of normal probability plot was used to assess deviations for normality. These were:

(a) SCE (raw data normally distributed, so no transformation was deemed necessary).

(b) CRABNOR (chromosome aberrations per cell).

(c) CRABLOG = Log_{10} (100*CRABNOR = 1). This was used because the raw data on chromosome aberrations were skewed to the right. The transformation successfully normalized the data.

Thus 18 multiple regressions were run (3 dependent variables times 3 ways of measuring arsenic exposure times 2 ways of measuring coffee consumption).

EVALUATION OF THE DATA

The raw data on CRABNOR and CRABLOG in relation to arsenic content in drinking water are reproduced as scattergraphs in figures 7 and 8, respectively. A comparison of chromosome aberrations in the control and exposed populations is presented in Figures 9 and 10.

A total of 19,264 cells were analyzed from lymphocyte preparations obtained from 104 cases in the exposed population. A total of 18,240 cells were studied from 86 control individuals. These average out to be about 189 cells/individual in exposed population and 192 cells/individual in the control. These samples are sufficiently large to yield reliable estimates of aberration frequencies. The frequency of chromosome aberrations turned out to be 0.033/cell for the control group and 0.026/cell for the exposed population.

For study of SCE's a total of 98 individuals was studied from the exposed population and a sample of 83 individuals in the control group. A scattergraph of raw data for the entire population is given in Figure 11. A comparison for the data on SCE's shows that the frequency of SCE's in the control group is similar to the frequency in various groups of the exposed population (Figure 12). An overall average of 8.301 SCE's in the control does not differ significantly from an overall frequency of 8.306 SCE's/cell in the exposed group.

A set of simple correlation coefficients between all pairs of variables is produced in Table 4. The data were so analyzed to find out if any of the covariates considered in the study had any effect on the frequency of aberrations and SCE's. This was carried out since agents like caffeine do induce chromosome aberrations in tissue culture of mammalian cells and in plants (see, e.g. Kihlman, 1976) even though its effect on this parameter is not evident when consumed as a beverage and ingested by the whole body. Table 5, summarizes the relationship between various parameters and genetic damage.

The six multiple regressions using SCE as a dependent variable were all statistically significant, but only 11.1% - 13.6% of the total variance in SCE was explained by the 10 independent variables as a group. Of the independent variables, CIGSGRP was significant in all six cases (smokers have more SCE's than non-smokers). The two regressions using ARSWAT as the measure of arsenic exposure were marginally significant for that variable (t for the regression coefficient = 2.044, $p = 0.042$, with COFFEE; $t = 2.041$, $p = 0.043$, with LOGCOFF). An increase in arsenic exposure as measured by ARSWAT leads to a modest increase in SCE frequency, holding other variables constant. However, this does not appear for ARSENIC concentration in water, or ARSGRP as defined by USEPA standards. Because of this, and because there is almost certainly considerable error in volunteers' estimates of their daily water consumption, the results with ARSWAT should be viewed skeptically. The only variable measured in this study which clearly increases SCE frequency is cigarette use.

The six regressions using CRABNOR as a dependent variable were all statistically significant, with 10.9% - 13.0% of the variance in CRABNOR being explained by the 10 independent variables as a group. CITYGRP was significant in all 6 cases, and COFFEE in the three regressions in which it was used. With CRABLOG as the dependent variable, only the three regressions with COFFEE were significant. (The three with LOGCOFF were not). In the three significant regressions, however, the only significant independent variable was again CITYGRP. The urban Nevadans in this sample exhibit higher frequencies of chromosome aberrations than the rural Nevadans, other things being equal, and there is some indication that coffee consumption may increase chromosome aberration frequency. However, arsenic exposure has no relationship with chromosome aberrations in this sample.

Further Analysis of Data by Project Officer

The data for twenty-two participants with reported exposures to pesticides, smelters, and/or chemicals were excluded from this analysis due to the unknown effects of these exposures on cytogenetic outcome measures. Since the arsenic exposed participant population was significantly older than the comparison population,the sample was divided into three age strata (<20 years, >20 and <50 years, and >50 years). Multiple regression analysis was used to test the relationship(s) of sister chromatid exchanges and chromosome aberrations with levels of arsenic in drinking water (or intake of waterborne arsenic) and covariates sex; age; coffee, wine, and cigarette consumption; and residence location (rural or urban). None of the regressions for the first age stratum (<20 years) explained a significant part of the variations in sister chromatid exchanges, chromosome aberrations, or log transformed chromosome aberrations; p values ranged from 0.6043 to 0.8436. Removal of the two outlier values with Student t Residuals of 3 or larger reduced the p values but none were significant (the smallest value was 0.2024). All of the regressions for the second stratum (>20 and <50 years) were significant (p values ranged from 0.0008 to 0.0220) but the coefficients for arsenic (either as level in water or amount consumed) were not significant (p values ranged from 0.2371 to 0.6426). Removal of one outlier with a student t residual greater than 3 increased the p values sufficiently that none of the regressions should be regarded as significant (p values ranged from 0.2003 to 0.3778). All of the regressions for age group >50 years were significant (p values ranged from 0.0003 to 0.0857) but the coefficients for arsenic were not significant (p values ranged from 0.1361 to 0.6959). Since the coefficient for residence location was significant for all of these regressions and residence location is strongly related to arsenic level there might be reason for concern that the arsenic effect is masked. However, since the coefficients for residence location are positive indicating a higher frequency of sister chromatid exchanges and chromosome aberrations in the urban areas which have low levels of waterborne arsenic, there does not appear to be any indication of an arsenic related effect.

CONCLUSIONS

This study compares data on cytogenetic parameters of two populations. One was 'exposed' to arsenic in drinking water to the extent of 2.18 times more than mandated by the current National regulations. The other group has been drinking water containing arsenic in quantities within those permitted by the USEPA. The data do not show any increase in the incidence of genetic damage (as measured by chromosome aberrations and SCE's) in the 'exposed' population. These studies, therefore, might indicate that there exists no evidence of genetic damage in human populations exposed to moderate quantities of arsenic in drinking water. In the present study, most individuals were exposed to 0.1 mg/l of total arsenic in drinking water and some had been consuming water containing twice as much, or even more, arsenic. The quantity of water consumed by this exposed group is also higher than in the control group since most of these individuals are farm, or out-door, workers. The summer temperature in the desert of Nevada coupled with very low humidity also creats larger demands for consumption of water.

The frequency of genetic aberrations is known to be higher in older individuals and very young children compared to adults in their twenties and thirties. If one considers these parameters alone the frequency of damaged cells in the exposed populations should have been higher. The lower frequency of such damage in exposed group may be attributed to the out-door life style of this group and to a minor degree, though questionable, to the drinking (wine) levels of the control group.

Even though evidence pointing to mutagencity and carcinogenicity of arsenic at relatively higher doses has been strongly suggested (Fishbein, 1981; Benko, 1977; Pershagen, 1981) and hearing loss in children has been reported (Benko and Seymon, 1977) the present data do not suggest such damage when arsenic is comsumed in modest quantities in water. Similar lack of health effect has been suggested by Southwick et al (1977) in the so-called Utah Report on the subject. Our studies, though not compiled for analysis of carcinogenic potential of arsenic, do not indicate an increased incidence of cancer in our exposed population. This is strongly suggested by a lack of increased incidence of SCE's by arsenic exposure in our population in view of the fact that direct, though only qualitative, correlation is found to exist between the induction of SCE's by a given agent and its ability to cause neoplasia (Perry and Evans, 1975). Also in our survey we did not come across any specific complaints about hearing loss.

In summary, the data presented here provide reasonable evidence that there is no indication of increased evidence of genetic damage as measured by chromosome aberrations and sister chromatid exchanges in the lymphocytes of subjects exposed for five years to 0.1 mg/l or slightly higher concentrations of arsenic in drinking water.

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TABLE I: DETAILED DATA ON POPULATION CHARACTERISTICS, CHROMOSOME DAMAGE AND SISTER CHROMATID EXCHANGES FOR EVERY INDIVIDUAL VOLUNTEER SUBJECT.

CASE	ID	ARSENIC PPM	CHROMOSOME ABERRATIONS PER CELL	NUMBER OF CELLS	SCE PER CELL	SEX	AGE	EXPOSURE TO PEST	SHELT	CHEM	WATER GLASSES PER DAY	COFFEE CUPS PER DAY	WINE GLASSES PER DAY	CIGARETTES PACKS PER DAY	HEALTH	CITY
001	001	0.015	0.130	200	08.50	F	47	N	N	N	05	25	0	1.5	0	HIDDEN VALLEY
002	002	0.015	0.075	200	09.10	M	52	N	N	N	03	08	1	0.0	0	HIDDEN VALLEY
003	003	0.015	0.090	200	07.96	M	17	N	N	N	04	00	0	0.0	0	HIDDEN VALLEY
004	004	0.005	0.050	200	10.40	M	24	N	N	N	02	04	0	0.0	0	RENO
005	005	0.005	0.065	200	08.58	M	21	N	N	N	01	00	0	0.0	0	RENO
006	006	0.005	0.075	200	10.76	F	53	N	N	N	03	03	0	0.0	0	RENO
007	007	0.005	0.050	200		M	48	N	N	N	04	02	0	0.0	0	RENO
008	008	0.050	0.060	200	11.08	F	52	N	N	N	05	03	0	1.0	1	RENO
009	009	0.050	0.070	200	09.42	M	76	N	N	N	02	02	0	0.0	1	RENO
010	011	0.065	0.055	200		F	39	N	N	N	07	05	2	0.0	0	HIDDEN VALLEY
011	012	0.065	0.045	200	06.60	F	13	N	N	N	08	00	0	0.0	0	HIDDEN VALLEY
012	013	0.065	0.052	133		M	09	N	N	N	00	00	0	0.0	0	HIDDEN VALLEY
013	014	0.065	0.055	200		F	07	N	N	N	04	00	0	0.0	0	HIDDEN VALLEY
014	015	0.005	0.040	200	09.22	F	31	N	N	N	04	03	1	0.0	0	RENO
015	017	0.005	0.020	200		F	18	N	N	N	04	00	0	0.0	0	RENO
016	031	0.100	0.130	200	09.62	M	51	N	N	N	08	04	0	2.0	0	FALLON
017	032	0.085	0.060	200	08.50	M	25	N	N	N	09	12	3	0.0	0	FALLON
018	033	0.005	0.075	200	10.19	M	21	N	N	N	05	02	0	0.0	0	RENO
019	034	0.005	0.080	200	09.29	F	20	N	N	N	02	02	1	0.0	0	RENO
020	035	0.005	0.050	200	10.00	M	25	N	N	N	02	03	1	0.0	0	RENO
021	036	0.005	0.055	200	08.46	M	20	N	N	N	05	01	0	0.2	0	RENO
022	037	0.005	0.035	200	08.68	M	20	N	N	N	06	02	0	0.0	0	RENO
023	039	0.100	0.050	200		M	56	Y	N	N	03	02	0	0.0	1	FALLON
024	040	0.100	0.045	200	09.38	F	49	N	N	N	02	06	0	0.0	1	FALLON
025	041	0.085	0.045	200	08.94	F	19	N	N	N	04	01	0	0.0	0	FALLON
026	042	0.005	0.045	200	07.02	M	21	N	N	N	08	10	0	0.0	0	RENO
027	043	0.005	0.045	200	08.50	M	21	N	N	N	06	02	0	0.5	0	RENO
028	044	0.005	0.025	200	07.48	F	22	N	N	N	02	03	0	0.2	0	RENO
029	045	0.095	0.035	200	08.04	F	42	N	N	N	07	03	0	0.0	0	FALLON
030	046	0.095	0.050	200	07.52	M	42	N	Y	N	04	08	0	0.0	0	FALLON

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TABLE I: continuous ...

031	047	0.095	0.050	200	06.50	M	17	N	N	N	07	01	0	0.0	0	FALLON
032	048	0.255	0.020	200	08.88	F	59	N	N	N	07	02	1	0.0	0	FALLON
033	049	0.255	0.025	079		M	59	Y	N	N	07	03	0	0.0	0	FALLON
034	050	0.050	0.040	200	08.45	F	44	N	N	N	08	10	0	0.0	0	FERMLEY
035	051	0.050	0.025	200	07.84	F	35	N	N	N	02	03	2	0.0	0	FERMLEY
036	052	0.040	0.060	200	08.05	F	47	N	N	N	03	05	0	1.0	0	FERMLEY
037	053	0.040	0.035	200	10.33	M	49	N	N	N	05	07	1	0.0	0	FERMLEY
038	054	0.005	0.050	200	08.92	M	23	N	N	N	04	02	9	0.0	0	RENO
039	055	0.005	0.025	200	10.00	M	26	N	N	N	03	10	0	1.0	0	RENO
040	056	0.005	0.025	200	08.60	M	20	N	N	N	09	03	1	0.0	0	RENO
041	057	0.005	0.025	200	09.44	M	26	N	N	N	02	00	0	0.0	1	RENO
042	058	0.005	0.010	200	09.50	M	20	N	N	N	03	01	0	0.0	0	RENO
043	059	0.005	0.030	200		F	20	N	N	N	02	02	0	0.0	0	RENO
044	023	0.005	0.040	200	06.68	M	22	N	N	N	02	04	0	0.0	0	RENO
045	060	0.080	0.015	200	09.19	M	45	N	N	N	03	03	0	1.0	0	FALLON
046	061	0.010	0.030	200	08.46	M	26	N	N	N	06	05	0	0.0	0	FALLON
047	062	0.010	0.035	200	07.86	F	21	N	N	N	01	02	0	0.0	0	FALLON
048	064	0.015	0.010	200	07.98	M	62	N	N	N	06	02	0	0.0	0	FALLON
049	065	2.275	0.015	200	09.32	F	45	N	N	N	03	04	0	0.0	0	FALLON
050	066	0.010	0.050	200	08.04	F	49	N	N	N	04	03	0	0.0	0	FALLON
051	068	0.195	0.025	200	07.94	M	59	Y	N	N	05	05	0	0.0	1	FALLON
052	069	0.195	0.010	200	08.98	F	58	N	N	N	07	04	0	0.0	1	FALLON
053	070	0.045	0.030	200	08.66	M	53	N	N	N	07	04	0	0.0	1	FALLON
054	071	0.045	0.045	200	08.56	F	51	N	N	N	03	07	0	0.0	0	FALLON
055	072	0.080	0.040	200	08.98	F	53	N	N	N	03	05	0	0.0	1	FALLON
056	073	0.080	0.030	200	07.86	M	54	Y	N	N	05	04	0	0.0	1	FALLON
057	074	0.010	0.020	100	07.82	M	29	N	N	Y	08	04	0	0.0	1	FALLON
058	075	0.275	0.030	100	07.88	M	55	Y	Y	N	03	03	0	0.0	1	FALLON
059	076	0.170	0.035	200	08.16	M	42	N	N	N	07	06	0	0.0	0	FALLON
060	077	0.170	0.015	200	09.52	F	41	N	N	N	00	03	1	0.0	1	FALLON
061	067	0.275	0.030	200	08.72	F	58	N	N	N	04	05	0	1.0	1	FALLON
062	078	0.170	0.020	200	07.70	M	10	N	N	N	08	00	0	0.0	0	FALLON
063	079	0.170	0.040	200	08.28	F	17	N	N	N	03	00	0	0.0	0	FALLON
064	080	0.170	0.050	200	07.84	M	25	Y	N	N	14	04	0	0.0	1	FALLON
065	081	0.010	0.040	100	10.08	M	60	N	Y	N	10	13	0	1.5	0	FALLON
067	083	0.050	0.030	200	09.44	M	42	N	N	N	03	03	0	1.5	0	FALLON
068	084	0.050	0.010	200	07.80	F	56	N	N	N	03	02	0	1.0	0	FALLON
069	085	0.045	0.025	200	08.22	F	22	N	N	N	02	07	0	1.0	0	FALLON
070	086	0.045	0.030	200	07.78	M	20	N	N	N	11	03	0	0.2	0	FALLON

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TABLE I: continuous ...

071	087	0.005	0.040	100	08.08	F	21	N	N	N	02	02	0	0.0	0	RENO
072	088	0.005	0.035	200	07.76	F	22	N	N	N	03	02	0	0.0	0	RENO
073	089	0.035	0.045	200	09.68	F	28	N	N	N	02	05	0	1.0	0	FALLON
074	090	0.035	0.040	200	11.72	F	10	N	N	N	03	00	0	0.0	0	FALLON
075	091	0.035	0.030	200	07.72	F	55	N	N	N	02	03	0	0.0	1	FALLON
076	100	0.060	0.005	200	08.92	M	44	N	N	N	03	04	1	0.0	0	FALLON
077	101	0.000	0.030	200	08.20	M	36	N	N	N	03	02	0	0.0	0	CINCINNATI
078	102	0.000	0.030	200	07.72	M	48	N	N	N	06	03	1	0.0	0	CINCINNATI
079	103	0.005	0.045	200	05.84	F	26	N	N	N	03	00	0	0.0	0	RENO
080	104	0.010	0.035	200	06.88	F	42	N	N	N	05	03	0	0.0	0	FALLON
081	105	0.140	0.025	200	06.40	F	34	N	N	N	02	02	0	0.0	0	FALLON
082	106	0.140	0.030	200	07.16	M	18	N	N	N	06	05	0	0.0	1	FALLON
083	107	0.005	0.020	200	08.92	F	41	N	N	N	04	04	0	0.0	0	RENO
084	108	0.055	0.045	200	07.76	F	41	N	N	N	02	00	0	1.0	1	FALLON
085	109	0.055	0.040	200	06.88	M	18	N	N	N	03	00	0	0.0	0	FALLON
086	110	0.005	0.015	260	05.84	F	16	N	N	N	05	01	0	0.0	0	RENO
087	076	0.170	0.030	200	06.44	M	42	N	N	N	07	06	0	0.0	0	FALLON
088	078	0.170	0.015	200	06.76	M	10	N	N	N	08	00	0	0.0	0	FALLON
089	080	0.170	0.050	200	09.88	M	23	Y	N	N	14	04	0	0.0	1	FALLON
090	092	0.005	0.040	200	06.12	M	24	Y	N	N	08	00	0	0.0	0	FALLON
091	093	0.005	0.030	200	09.36	F	20	N	N	N	01	02	0	0.0	1	RENO
092	111	0.380	0.045	200	07.88	M	48	N	N	N	02	03	0	0.0	0	RENO
093	094	0.005	0.025	200	07.04	M	23	N	N	N	03	03	1	0.0	0	FALLON
094	095	0.005	0.020	200	07.44	M	25	N	N	N	04	04	0	0.0	0	RENO
095	096	0.005	0.020	200	08.92	M	25	N	N	N	05	00	0	0.0	0	RENO
096	097	0.005	0.025	200	08.24	M	21	N	N	N	06	03	0	0.0	0	RENO
097	098	0.005	0.005	200	10.16	F	21	N	N	N	03	02	0	0.0	0	RENO
098	099	0.005	0.015	200	08.28	F	47	N	N	N	02	03	0	0.0	0	RENO
099	112	0.005	0.020	200	07.48	F	30	N	N	N	03	06	0	0.0	1	RENO
100	113	0.005	0.025	200	07.92	M	26	N	N	N	02	05	3	0.0	0	RENO
101	114	0.115	0.040	200	08.76	F	22	Y	N	N	15	00	1	0.0	1	VIRGINIA FOOTHILLS
102	115	0.005	0.030	200	10.64	F	22	N	N	N	04	02	0	0.0	0	RENO
103	116	0.005	0.010	100	08.12	M	20	N	N	N	08	01	0	0.0	1	RENO
104	118	0.005	0.000	090	06.92	M	19	N	N	N	09	01	0	0.0	1	RENO
105	119	0.005	0.010	200	06.56	M	19	N	N	N	04	02	0	0.0	0	RENO
106	120	0.095	0.025	200	06.88	M	41	N	N	N	09	02	1	0.0	0	FALLON
107	121	0.095	0.010	200	07.76	F	35	N	N	Y	08	03	0	0.0	0	FALLON
108	122	0.045	0.015	200	07.20	M	51	Y	N	N	03	05	1	0.0	0	FALLON
109	123	0.020	0.050	200	07.76	M	80	N	N	N	07	00	0	0.0	0	FALLON
110	124	0.020	0.015	200	09.00	F	75	N	N	N	04	00	0	0.0	1	FALLON

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TABLE I: continuous ...

111	125	0.045	0.005	200	09.28	F	43	N	N	N	04	05	1	0.0	0	FALLON
112	126	0.045	0.015	200	07.88	F	17	N	N	N	05	01	1	0.0	1	FALLON
113	127	0.100	0.010	200	08.28	F	35	N	N	N	08	05	0	1.0	0	FALLON
114	128	0.100	0.015	200	07.72	M	13	N	N	N	02	01	0	0.0	0	FALLON
115	129	0.100	0.010	200		M	14	N	N	N	03	00	0	0.0	0	FALLON
116	130	0.100	0.020	200	06.72	F	70	N	N	N	00	02	0	0.0	0	FALLON
117	131	0.110	0.020	200	07.84	M	22	N	N	N	05	01	0	0.0	0	FALLON
118	132	0.100	0.010	200	08.16	F	38	Y	N	N	02	10	0	1.5	1	FALLON
119	133	0.095	0.060	200	09.88	F	32	N	N	N	08	02	0	0.0	1	FALLON
120	134	0.095	0.025	200	06.72	M	41	N	N	N	06	04	0	0.0	0	FALLON
121	135	0.095	0.000	200	08.28	F	10	N	N	N	07	00	0	0.0	0	FALLON
122	136	0.015	0.025	200	06.84	M	15	Y	N	N	09	00	0	0.0	0	FALLON
123	137	0.015	0.035	200	06.76	M	11	N	N	N	04	00	0	0.0	0	FALLON
124	138	0.100	0.010	200	06.80	M	37	N	N	N	03	05	0	0.0	1	FALLON
125	139	0.005	0.030	200	06.52	M	27	N	N	N	07	04	1	0.0	1	RENO
126	140	0.005	0.025	200	07.56	F	26	N	N	N	01	03	0	0.0	0	RENO
127	141	0.005	0.033	150	06.80	F	44	N	N	N	03	02	1	0.0	0	RENO
128	142	0.100	0.040	200	09.04	M	62	N	N	N	02	04	0	0.0	1	FALLON
129	143	0.035	0.010	200	07.08	F	32	N	N	N	05	03	0	0.0	0	FALLON
130	144	0.035	0.010	200	05.92	M	32	Y	N	N	06	03	0	0.0	0	FALLON
131	145	0.100	0.020	200	07.60	M	14	N	N	N	01	00	0	0.0	0	FALLON
132	146	0.100		000	11.96	M	49	N	N	N	06	13	0	2.0	0	FALLON
133	147	0.100	0.030	100	10.88	F	40	N	N	N	08	02	0	0.0	0	FALLON
134	148	0.100	0.025	200	07.45	F	44	N	N	N	05	02	0	0.5	1	FALLON
135	149	0.100	0.015	200	05.88	M	14	N	N	N	02	02	0	0.0	0	FALLON
136	150	0.100	0.010	200	06.84	M	44	N	N	N	03	01	0	0.0	0	FALLON
137	151	0.100	0.015	200	07.36	M	58	N	N	N	04	06	0	2.0	0	FALLON
138	152	0.100	0.010	200	08.48	F	55	N	N	N	00	03	0	0.0	1	FALLON
139	153	0.100	0.025	200		M	40	N	N	N	08	05	0	0.0	0	FALLON
140	154	0.100	0.020	200	10.20	F	46	N	N	N	07	00	0	0.0	1	FALLON
141	155	0.100	0.010	200	09.04	M	18	N	N	N	09	00	0	0.0	0	FALLON
142	156	0.100	0.050	100	07.00	M	63	N	N	N	02	10	0	0.0	1	FALLON
143	157	0.100	0.005	200	06.16	F	57	N	N	N	01	12	0	0.0	0	FALLON
144	158	0.100	0.000	100	07.52	M	64	N	N	N	07	04	0	0.0	0	FALLON
145	159	0.100	0.020	200	07.80	M	64	N	N	N	03	05	0	0.0	0	FALLON
146	160	0.100	0.020	200	07.16	F	35	N	N	N	02	01	0	0.0	0	FALLON
147	161	0.100	0.025	200	07.44	M	36	N	N	N	05	07	0	0.0	0	FALLON
148	162	0.100	0.005	200	08.68	F	50	N	N	N	01	06	0	0.0	0	FALLON
149	163	0.100	0.015	200	07.64	M	47	Y	N	N	12	06	0	0.0	0	FALLON
150	164	0.100	0.005	200	07.24	M	42	N	N	N	08	00	0	0.0	0	FALLON

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TABLE I: continuous .....

151	165	0.100	0.010	200	07.32	M	69	N	N	N	08	00	0	0.0	0	FALLON
152	166	0.100	0.020	200	09.80	M	53	N	N	N	06	10	0	0.0	0	FALLON
153	167	0.100	0.005	200	09.28	F	46	N	N	N	07	03	0	0.0	1	FALLON
154	168	0.100	0.030	150	08.88	F	39	N	N	N	04	03	0	0.0	0	FALLON
155	169	0.100	0.020	200	06.80	F	51	N	N	N	03	03	0	0.0	0	FALLON
156	170	0.100	0.010	200	07.40	M	65	Y	N	N	01	04	0	0.0	1	FALLON
157	171	0.100	0.010	200	08.16	F	51	N	N	N	03	06	0	1.0	1	FALLON
158	172	0.100	0.020	200	07.40	M	51	N	Y	Y	03	05	0	0.0	0	FALLON
159	173	0.005	0.010	200	08.04	F	18	N	N	N	04	01	1	0.0	1	RENO
160	174	0.005	0.000	200	06.40	F	21	N	N	N	02	04	1	0.2	0	RENO
161	175	0.005	0.010	200	09.24	M	23	N	N	N	03	02	0	0.0	0	RENO
162	176	0.005	0.010	200	09.56	M	30	N	N	N	04	04	0	1.0	1	RENO
163	177	0.005	0.030	200	08.20	M	19	N	N	N	04	07	1	0.0	0	RENO
164	178	0.005	0.020	200	08.92	M	28	N	N	N	05	08	5	0.5	0	RENO
165	179	0.005	0.015	200	07.92	M	19	N	N	N	10	01	0	0.0	1	RENO
166	180	0.100	0.035	200	15.28	F	33	N	N	N	03	13	0	1.5	1	FALLON
167	181	0.100	0.010	200	09.08	F	51	N	N	N	03	05	0	0.0	1	FALLON
168	182	0.100	0.010	200	09.60	F	61	N	N	N	06	04	0	0.0	0	FALLON
169	183	0.100	0.005	200	07.52	M	34	N	N	N	06	05	0	0.0	0	FALLON
170	184	0.100	0.005	200	07.68	M	52	N	N	N	05	12	0	0.0	1	FALLON
171	185	0.100	0.006	179	08.56	M	62	N	N	N	08	08	0	0.0	0	FALLON
172	186	0.100	0.030	100	10.90	F	56	N	N	N	02	07	0	0.0	1	FALLON
173	187	0.100	0.025	200	09.00	F	43	N	N	N	35	02	1	0.0	0	FALLON
174	188	0.100	0.035	200	07.04	F	73	N	N	N	07	02	0	0.0	1	FALLON
175	189	0.100	0.040	200	08.72	M	47	N	N	N	04	18	0	0.5	0	FALLON
176	190	0.100	0.025	200	06.88	F	43	N	N	N	05	05	2	0.0	0	FALLON
177	191	0.100	0.040	200	09.44	F	50	N	N	N	01	04	0	0.0	0	FALLON
178	192	0.100	0.005	200	08.12	F	62	N	N	N	04	04	0	0.0	1	FALLON
179	193	0.100	0.015	200	10.25	F	57	N	N	N	04	12	0	1.0	0	FALLON
180	194	0.100	0.015	200	08.84	M	35	N	N	N	05	06	0	0.0	0	FALLON
181	195	0.100	0.000	200	08.88	M	49	N	N	N	06	10	1	0.0	0	FALLON
182	196	0.100	0.020	200	07.84	F	47	N	N	N	05	03	0	0.0	1	FALLON
183	197	0.100	0.025	123	07.60	M	35	N	N	N	05	02	0	0.0	0	FALLON
184	198	0.100	0.015	200	07.44	M	34	N	N	N	06	01	0	0.0	0	FALLON
185	199	0.100	0.020	100	06.68	M	48	N	N	N	10	06	0	0.0	0	FALLON
186	200	0.100	0.005	200	11.20	M	23	N	N	N	10	02	1	0.0	0	FALLON
187	201	0.100	0.050	200	07.88	M	53	N	N	N	03	06	0	0.0	0	FALLON
188	202	0.005	0.040	200	08.52	M	28	N	N	N	03	00	0	0.0	0	RENO
189	203	0.005	0.040	200	07.80	M	30	Y	N	N	03	08	2	0.0	0	RENO
190	204	0.005	0.050	200	08.84	M	23	N	N	N	07	05	0	0.0	0	RENO

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TABLE I: continuous ...

191	205	0.005	0.025	200	08.84	M	21	N	N	N	07	03	0	0.0	1	RENO
192	206	0.005	0.030	200	08.44	F	29	N	N	N	03	03	2	0.0	1	RENO
193	207	0.005	0.035	200	07.68	F	19	Y	N	N	03	00	1	0.0	0	RENO
194	208	0.005	0.045	200	08.72	M	26	N	N	N	04	03	0	0.5	0	RENO
195	209	0.005	0.020	200	08.40	M	25	N	N	N	06	01	1	0.0	0	RENO
196	210	0.100	0.025	200	06.40	F	37	N	N	N	02	04	1	0.0	0	FALLON
197	211	0.100	0.020	200	07.48	M	13	N	N	N	03	00	0	0.0	0	FALLON

FOOTNOTES: (1) FOR EXPOSURE TO PESTICIDES, CHEMICALS, SMELTERS; N = NOT EXPOSED, Y = EXPOSED.
(2) FOR HEALTH; 0 = NO REPORTED SYMPTOMS OF ARSENIC POISONING, 1 = SYMPTOMS OF ARSENIC POISONING.

TABLE II. DATA ON THE POPULATION CHARACTERISTICS WITH REGARDS TO SOME VARIABLES USED IN THE STUDY

Variable	Classification	Number of individuals studied in the group: Control (%)	Exposed (%)	Total (%)
Sex (var. 7)	Females:	36 (41.9)	49 (46.7)	85 (44.5)
	Males:	50 (58.1)	56 (53.3)	106 (55.5)
	Total:	86	105	191
Pesticides (var. 9)	Not exposed:	80 (93.0)	95 (90.5)	175 (91.6)
	exposed:	6 (7.0)	10 (9.5)	16 (8.4)
	Total:	86	105	191
Smelter	Not exposed:	85 (98.8)	102 (97.1)	187 (97.9)
	exposed:	1 (1.2)	3 (2.9)	4 (2.1)
	Total:	86	105	191
Chemicals (var. 11)	Not exposed:	85 (98.8)	103 (98.1)	188 (98.4)
	exposed:	1 (1.2)	2 (1.9)	3 (1.6)
	Total:	86	105	191
Wine (var. 16)	No wine:	64 (74.4)	92 (87.6)	156 (81.7)
	wine:	22 (25.6)	13 (12.4)	35 (8.3)
	Total:	86	105	191
Cigaretts (var. 16)	Non-smokers:	72 (83.7)	89 (84.8)	161 (84.3)
	smokers:	14 (16.3)	16 (15.2)	30 (15.7)
	Total:	86	105	191
Residence (var. 18)	Rural:	27 (31.4)	97 (92.4)	124 (64.9)
	Urban:	59 (68.6)	8 (7.6)	67 (5.1)
	Total:	86	105	191

TABLE III: Summary of definitions and coding of variables used in statistical interpretation of the data

AGE = Age of the participant in years.

ARSENIC = Arsenic content of drinking water in ppm or mg/l.

ARSWAT = (ARSENIC) X (glasses of water drunk per day).

ARSGRP = Groups or populations of volunteers who have been drinking water containing 0.05 mg/l arsenic or more for five years (exposed) or those who have been drinking water far containing <0.05 mg/ml arsenic for 5 years (control); 0 = control, 1 = exposed.

CHEM = Exposure to potentially carcinogenic/mutagenic chemicals; 0 = not exposed, 1 = exposed.

CIGSGRP = Cigarette consumers and non smokers; 0 = non-smoker; 1 = smoker.

CITYGRP = Classification according to location of residence; 0 = rural Nevada (Fallon, Ferneley), 1 = urban Nevada (Reno, Hidden Valley, Virginia Foothills)

COFFEE = Coffee consumption in cups per day; 0 = non consumers, 1 = consumers.

CRABLOG = Log_{10} (100 X CRABNOR + 1).

CRABNOR = Chromosome aberrations per cell.

LOGCOFF = Log_{10} (COFFEE + 1).

PEST = Exposure to pesticides; 0 = not-exposed, 1 = exposed.

SCE = Sister chromatid exchanges per cell.

SEX = Sex; 0 = female, 1 = male.

SMELT = Ever worked or not in smeler plant(s): 0 = no exposure, 1 = exposure.

WINEGRP = Wine conusmption or lack of it: 0 = no wine consumption, 1 = 1 or more glasses/day.

TABLE IV A SET OF SIMPLE CORRELATION COEFFICIENTS BETWEEN ALL PAIRS OF VARIABLES (for definitions of codes see TABLE III)

		ARSENIC 2	ARSGRP 3	CRABNOR 4	CRABLOG 5	SCE 6	SEX 7	AGE 8	PEST 9	SMELT 10	CHEM 11
ARSENIC	2	1.0000									
ARSGRP	3	.7944	1.0000								
CRABNOR	4	-.1577	-.1945	1.0000							
CRABLOG	5	-.1474	-.2092	.9335	1.0000						
SCE	6	-.0267	-.0235	.1994	.1741	1.0000					
SEX	7	-.0528	-.0697	.0222	.0166	-.1451	1.0000				
AGE	8	.3790	.4054	-.0439	-.0842	.0643	-.1324	1.0000			
PEST	9	.1198	.0205	-.0118	.0144	-.1398	.1363	.0258	1.0000		
SMELT	10	.1400	.0648	.0438	.0701	-.0056	.1359	.1419	.0967	1.0000	
CHEM	11	.0092	.0341	-.0785	-.0699	-.0615	.0298	.0131	-.0380	.2747	1.0000
WATER	12	.0947	.1127	-.0330	-.0456	.0226	.1501	-.0474	.1286	.0082	.0568
ARSWAT	13	.6393	.5603	-.0919	-.0970	.0463	-.0043	.1755	.1734	.0245	.0150
COFFEE	14	.0814	.1084	.1670	.0848	.1718	.0275	.3180	-.0056	.1496	.0081
LOGCOFF	15	.1118	.1138	.0476	.0065	.1592	.0054	.3820	-.0219	.1421	.0495
WINEGRP	16	-.1353	-.1781	-.0066	-.0107	.0288	-.0230	-.1273	.0711	-.0732	-.0632
CIGSGRP	17	-.0422	-.0168	.1420	.0963	.2106	-.0927	.0565	-.0716	.0361	-.0574
CITYGRP	18	-.5734	-.6551	.2585	.2389	.0879	.0773	-.4328	-.0777	-.1087	-.0939

		WATER 12	ARSWAT 13	COFFEE 14	LOGCOFF 15	WINEGRP 16	CIGSGRP 17	CITYGRP 18
WATER	12	1.0000						
ARSWAT	13	.7395	1.0000					
COFFEE	14	-.0402	-.0052	1.0000				
LOGCOFF	15	-.0717	.0019	.8837	1.0000			
WINEGRP	16	.1035	.0367	.0220	.0762	1.0000		
CIGSGRP	17	-.0627	-.0778	.3259	.2716	-.1356	1.0000	
CITYGRP	18	-.1124	-.4026	-.1201	-.1314	.1927	.0038	1.0000

TABLE V. SUMMARY OF MULTIPLE LINEAR REGRESSION ANALYSES OF CHROMOSOME ABERRATIONS AND SISTER CHROMATID EXCHANGES AS FUNCTIONS OF ARSENIC EXPOSURE AND OTHER INDEPENDENT VARIABLES.

Dependent Variable	Standardized Regression Coefficients for Independent Variables[1]													F[1] (For overall regression)	r[1]
	ARSENIC	ARSGRP	ARSWAT	SEX	AGE	PEST	SMELT	CHEM	COFFEE	LOGCOFF	WINEGRP	CIGSGRP	CITYGRP		
SCE	.061			-.097	.047	-.119	.002	-.043	.151		.030	.168+	.134	2.23+	11.0
		.052		-.098	.049	-.114	.007	-.046	.149		.031	.167+	.134	2.21+	11.1
			.163+	-.102	.052	-.136	.010	-.046	.157		.011	.170+	.171	2.65*	13.1
	.057			-.093	.044	-.117	.006	-.050		.129	.025	.183+	.129	2.12+	11.0
		.054		-.094	.044	-.112	.011	-.052		.129	.026	.181+	.133	2.11+	11.0
			.164+	-.097	.046	-.134	.014	-.052		.139	.006	.185+	.169	2.55*	13.0
CRABNOR	-.013			-.003	.026	.035	.057	-.066	.162+		-.058	.065	.316*	2.62*	12.8
		-.007		-.003	.025	.033	.056	-.066	.163+		-.058	.065	.319*	2.62*	12.8
			.040	-.004	.023	.027	.057	-.065	.164+		-.062	.067	.339*	2.65*	13.0
	-.018			.009	.067	.030	.070	-.068		.028	-.042	.110	.311*	2.18+	10.9
		-.006		.009	.065	.028	.069	-.067		.028	-.042	.111	.317*	2.17+	10.9
			.033	.008	.063	.023	.070	-.066		.029	-.045	.113	.334*	2.19+	11.0
CRABLOG	-.014			-.020	-.010	.059	.096	-.071	.092		-.062	.041	.281*	1.94+	9.8
		-.043		-.021	-.007	.057	.094	-.072	.092		-.063	.040	.263*	1.96+	9.9
			.020	-.020	-.013	.054	.095	-.070	.093		-.063	.043	.296*	1.95+	9.8
	-.017			-.013	.014	.056	.103	-.072		.014	-.052	.068	.278	1.81	9.2
		-.043		-.014	.017	.054	.102	-.072		.013	-.054	.067	.262	1.83	9.3
			.016	-.013	.010	.052	.103	-.071		.015	-.053	.069	.293	1.81	9.2

[1]Significance levels indicated as follows: + $-0.01 < p < 0.05$, * - $p < 0.01$

A map of Nevada showing locations of Reno and Fallon, the area from where the study population was derived.

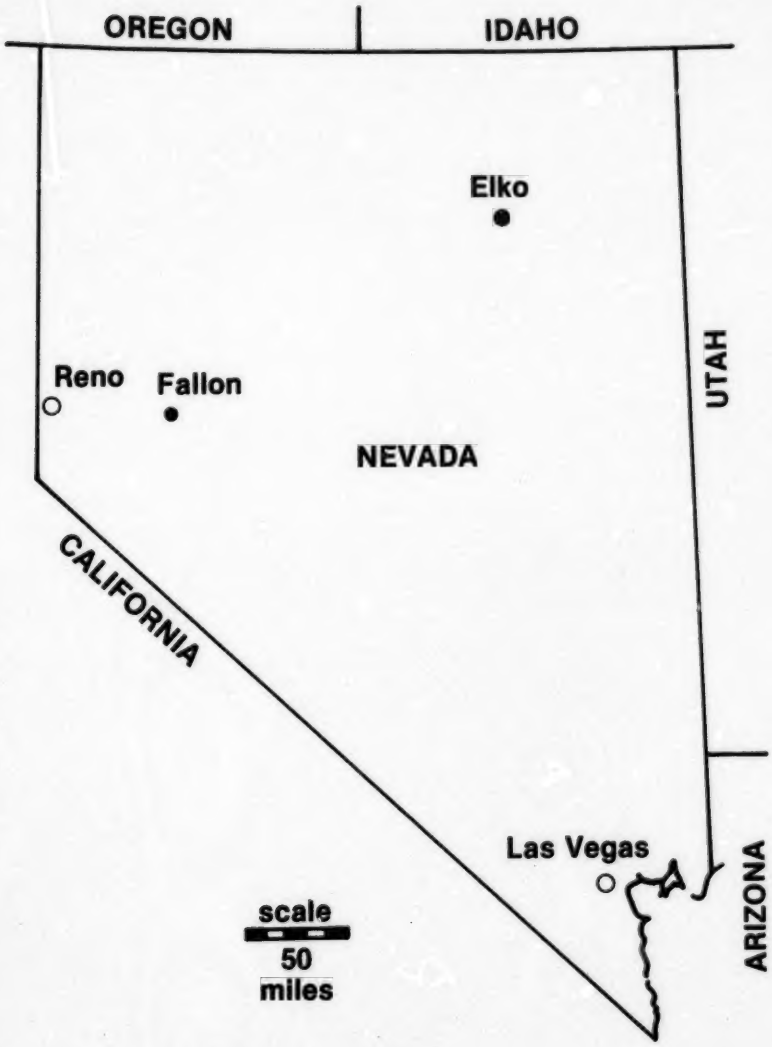


Figure 1.

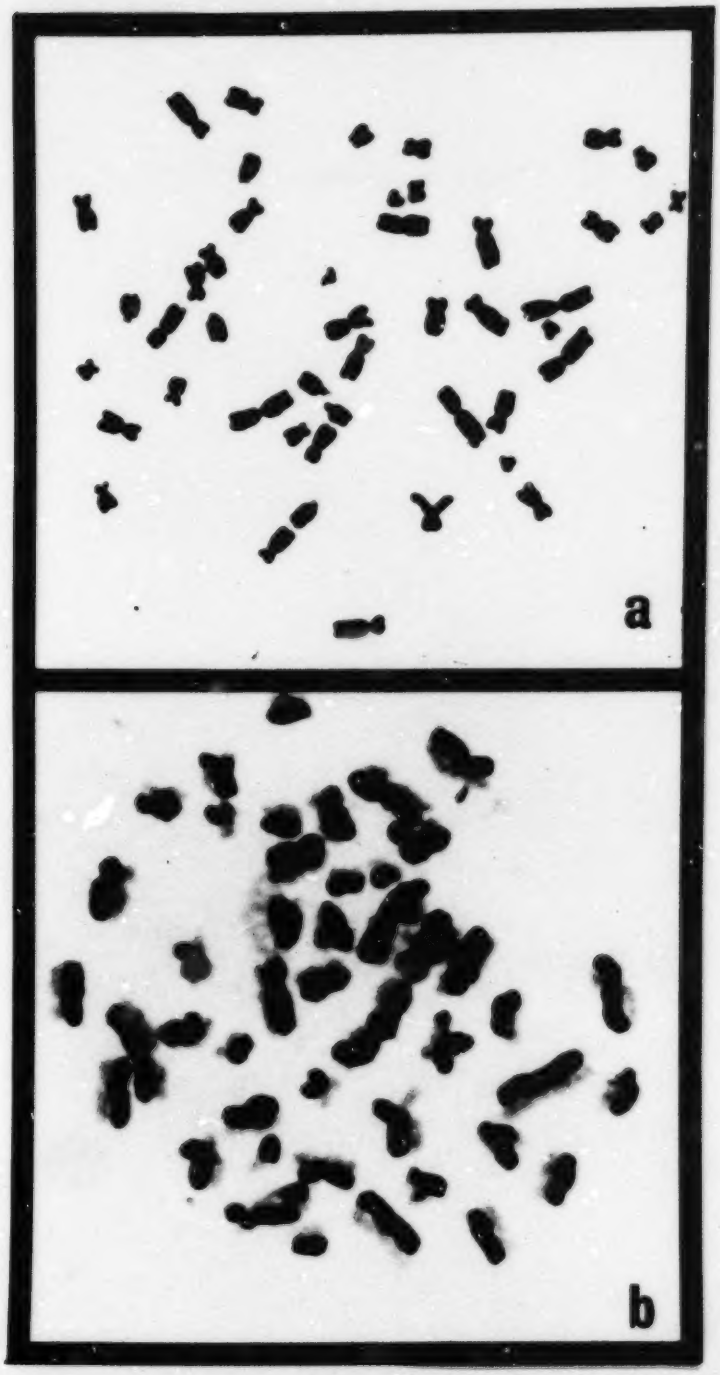
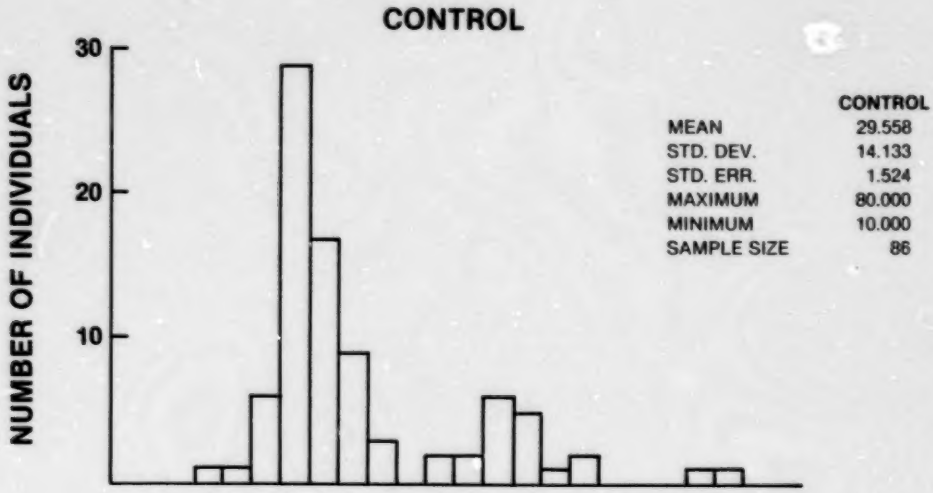
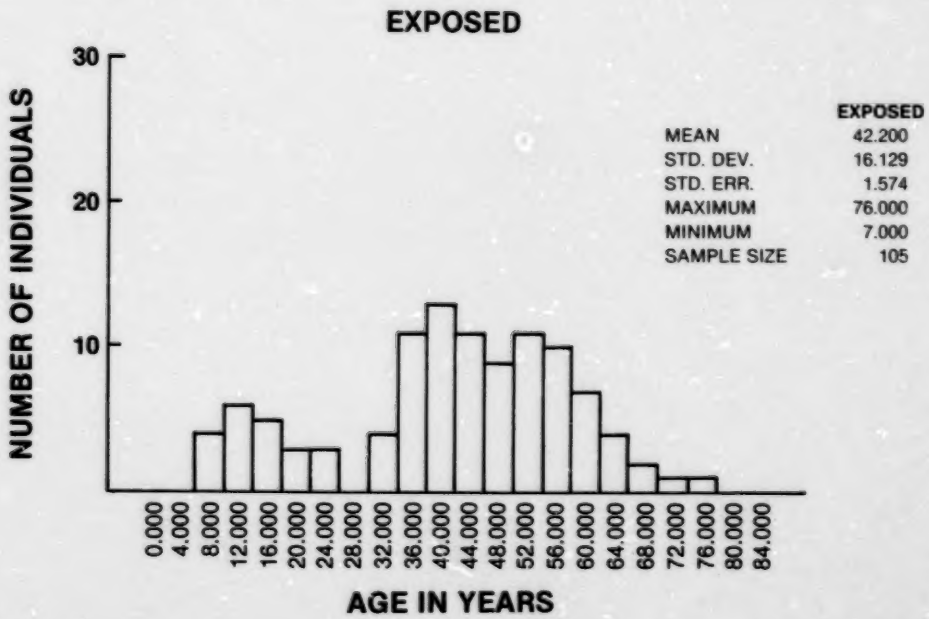


FIGURE 2. Examples of preparations used in our laboratory to study (a) chromosome aberrations (a normal cell from the first cell cycle, 48 hour lymphocyte culture) and (b) sister chromatid exchanges (BrdU incorporated for two cell cycles, 72 hour lymphocyte culture).

A histogram and related parameters of AGE distribution (var. 8) in the control and exposed population sample.



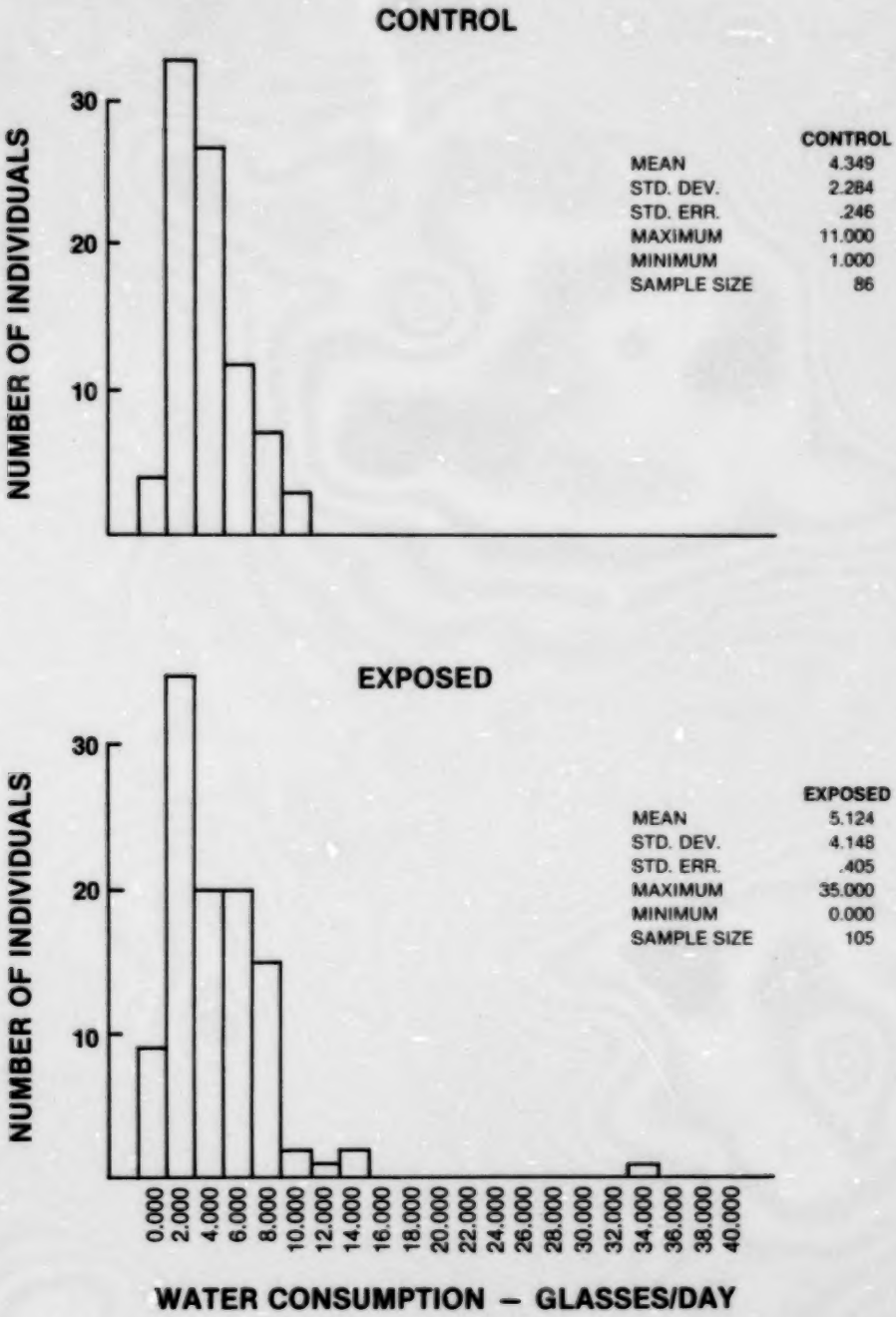


ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	TAIL PROBABILITY
BETWEEN GROUPS	7555.7289	1	7555.7289	32.43	.0000
WITHIN GROUPS	44034.0093	189	232.9842		
TOTAL	51589.7382	190			

FIGURE 3.

Histogram and related parameters of the values for WATER consumed/day (var. 12) by the control and exposed population.

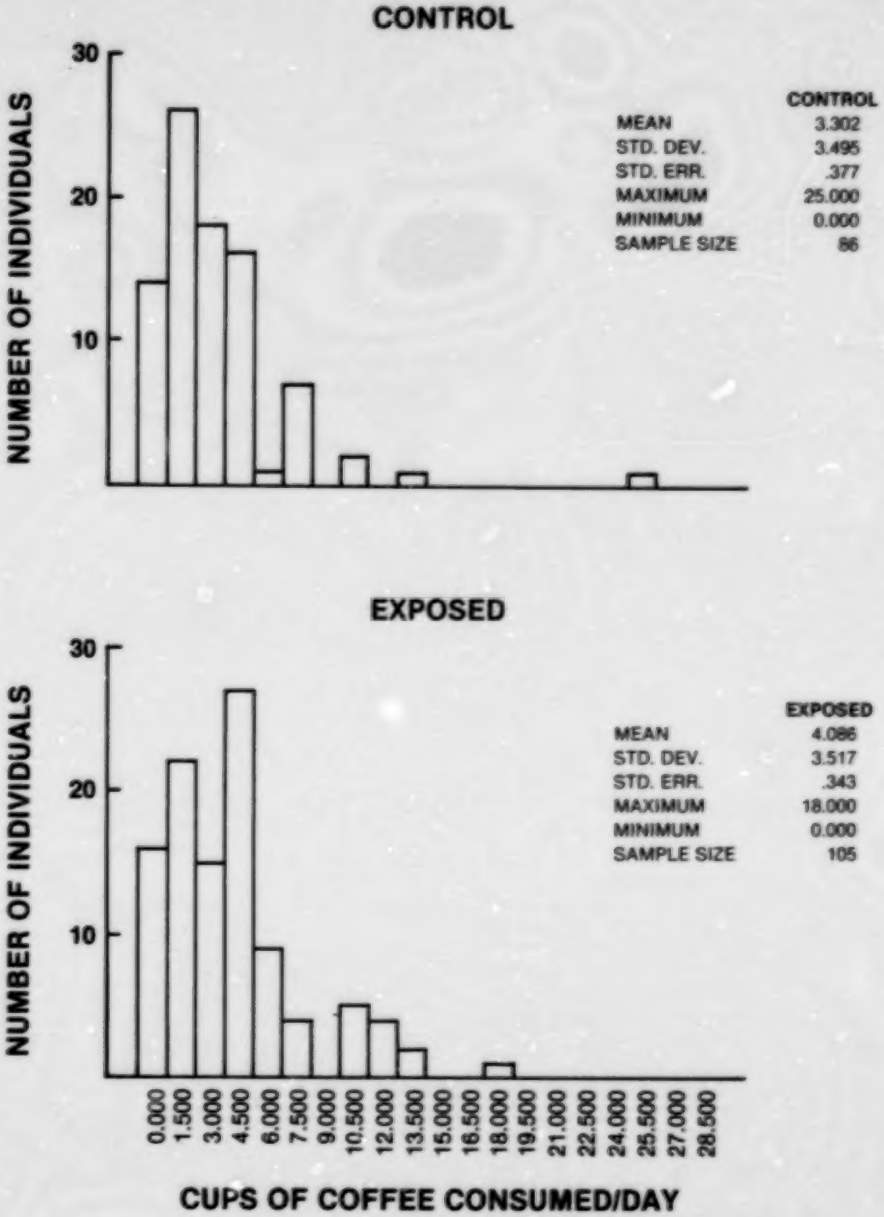


ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	TAIL PROBABILITY
BETWEEN GROUPS	28.3940	1	28.3940	2.40	.1227
WITHIN GROUPS	2232.9254	189	11.8144		
TOTAL	2261.3194	190			

FIGURE 4.

Histogram showing the amount of COFFEE consumed per day (var. 14) by the control and exposed sample population. Related parameters are given also.



ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	TAIL PROBABILITY
BETWEEN GROUPS	29.0141	1	29.0141	2.36	.1262
WITHIN GROUPS	2324.3681	189	12.2982		
TOTAL	2353.3822	190			

FIGURE 5.

Arsenic Exposure in control and exposed groups.

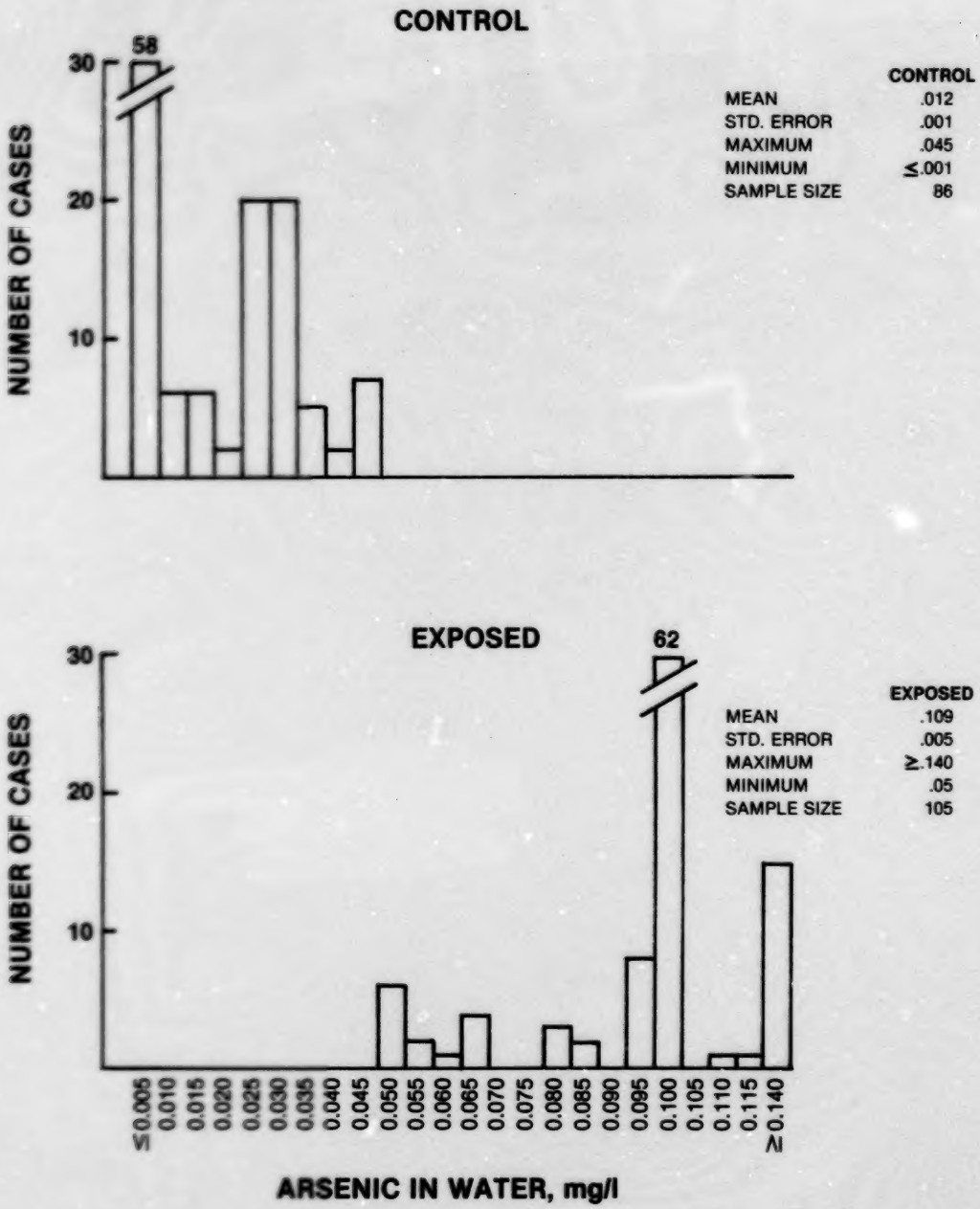


FIGURE 6.

A scattergraph showing the raw data on chromosome aberrations (CRABNOR) in relation to the ARSENIC concentration in drinking water of the sample population.

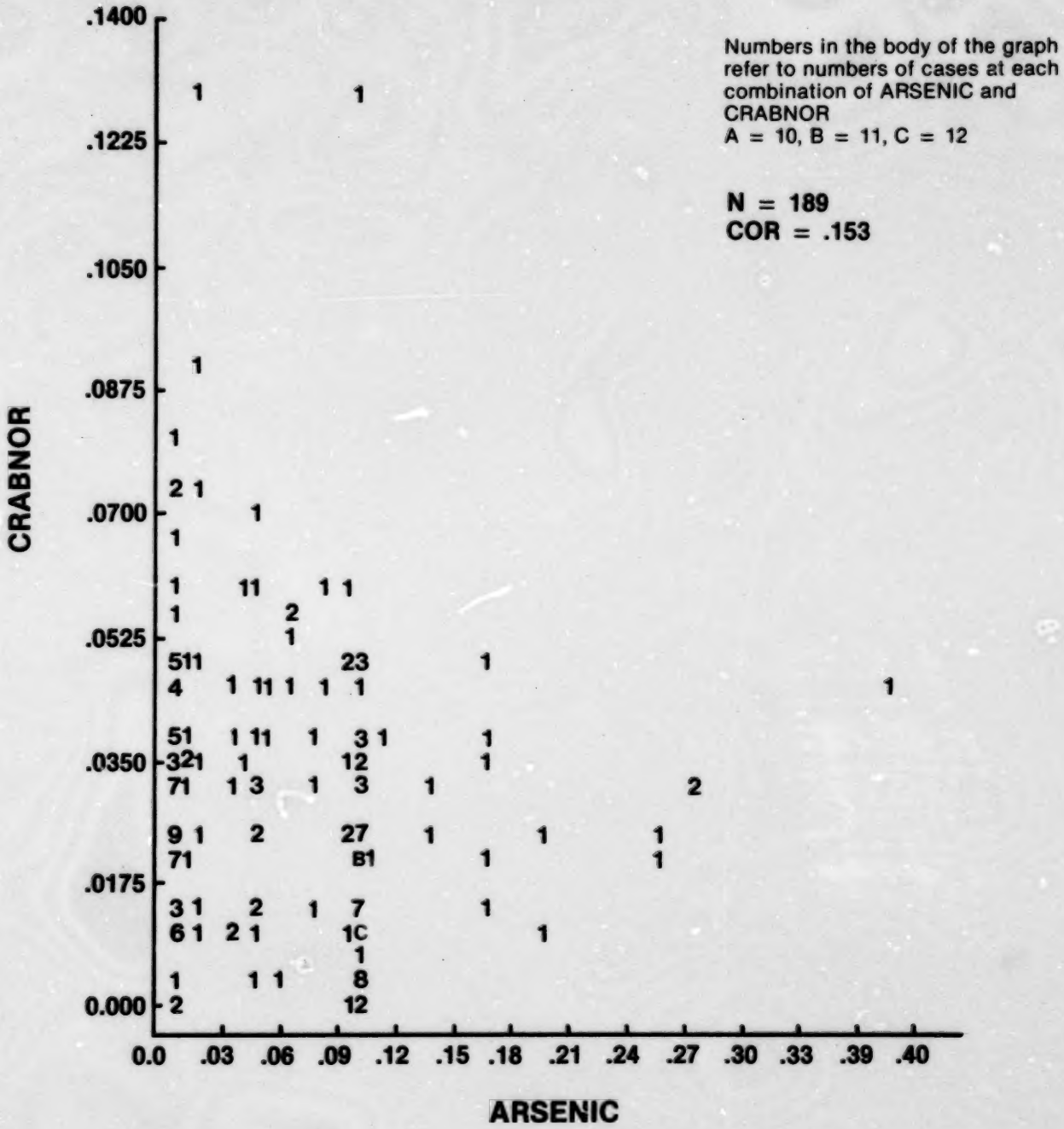


FIGURE 7.

A scattergraph showing CRABLOG in relation to the variable ARSENIC. See figure 7 for explanation of numbers.

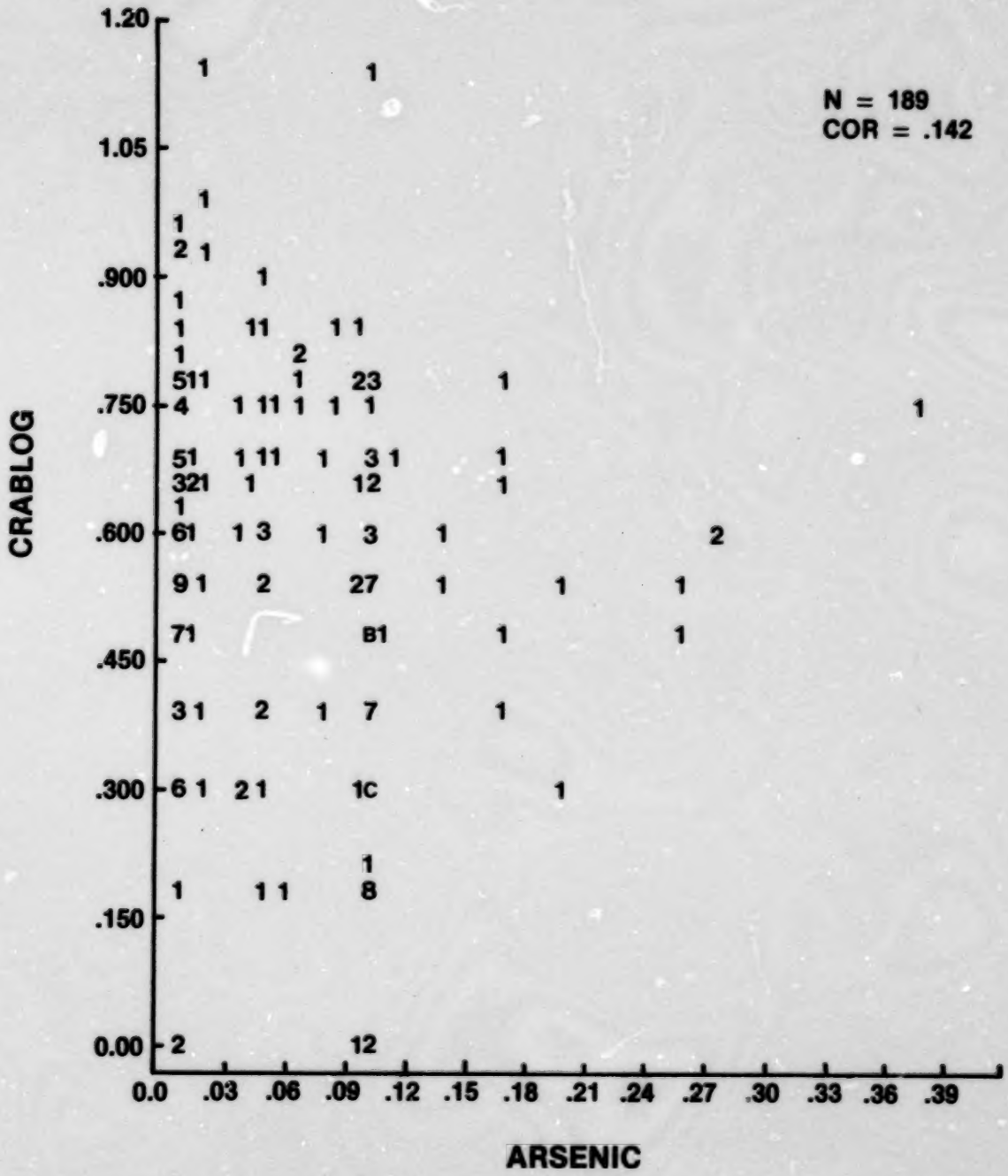
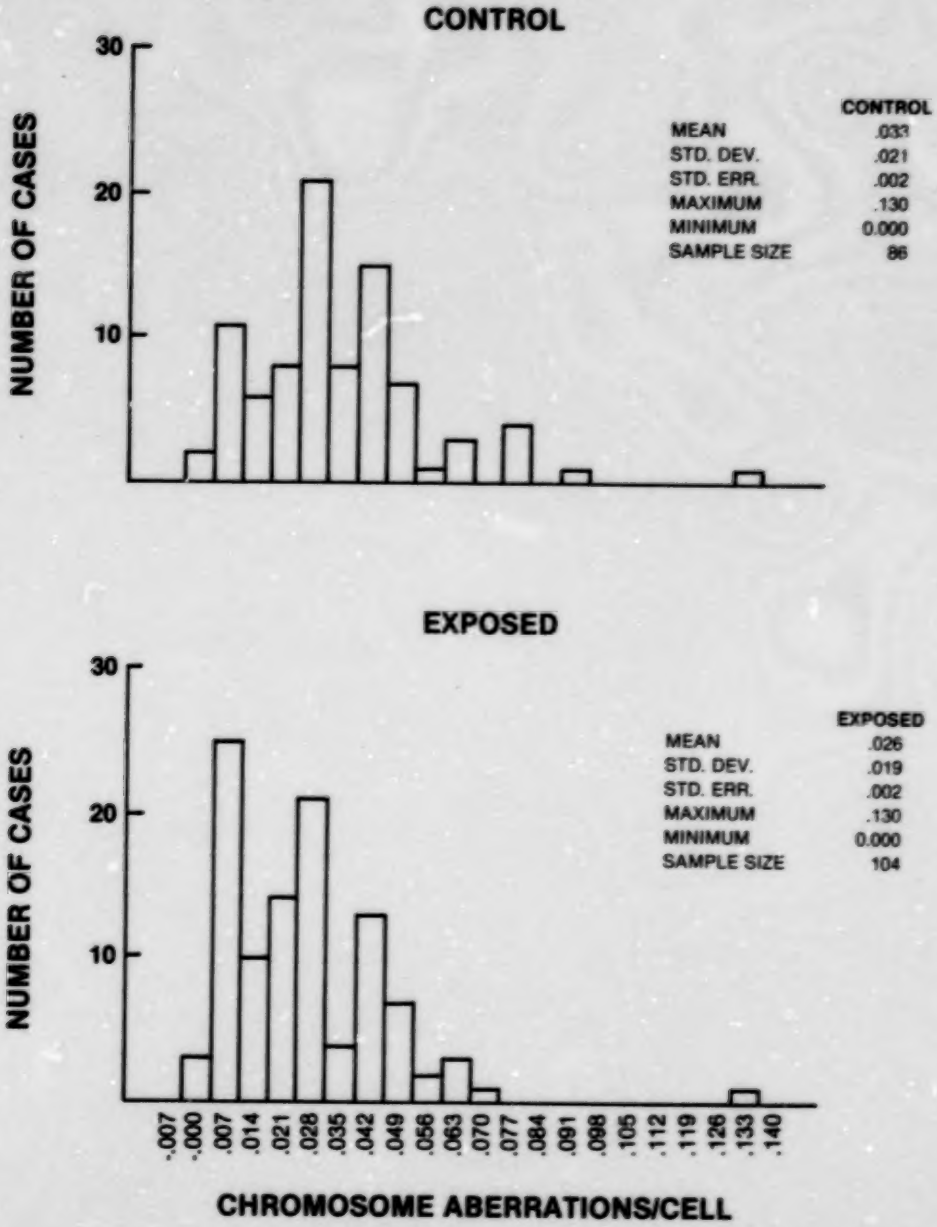


FIGURE 8.

Number of cases in control and exposed groups with various frequencies of chromosome aberrations per cell (var. 4).

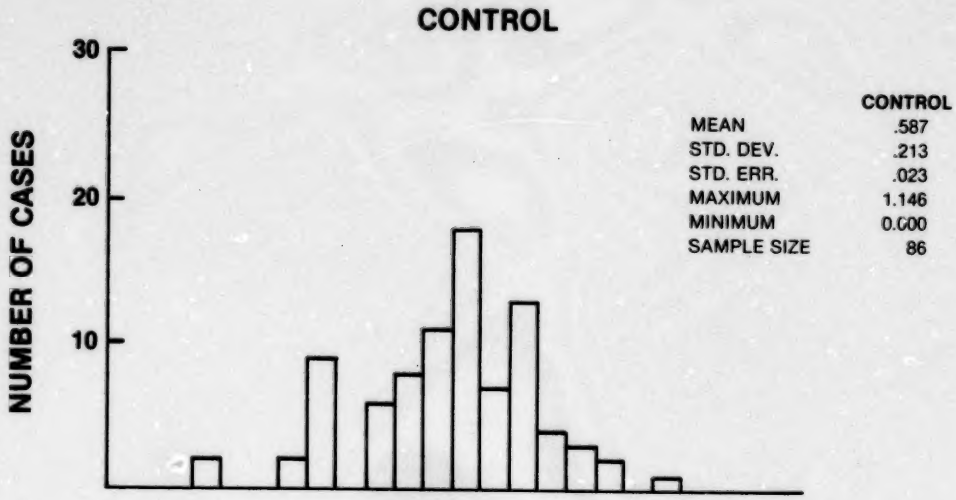


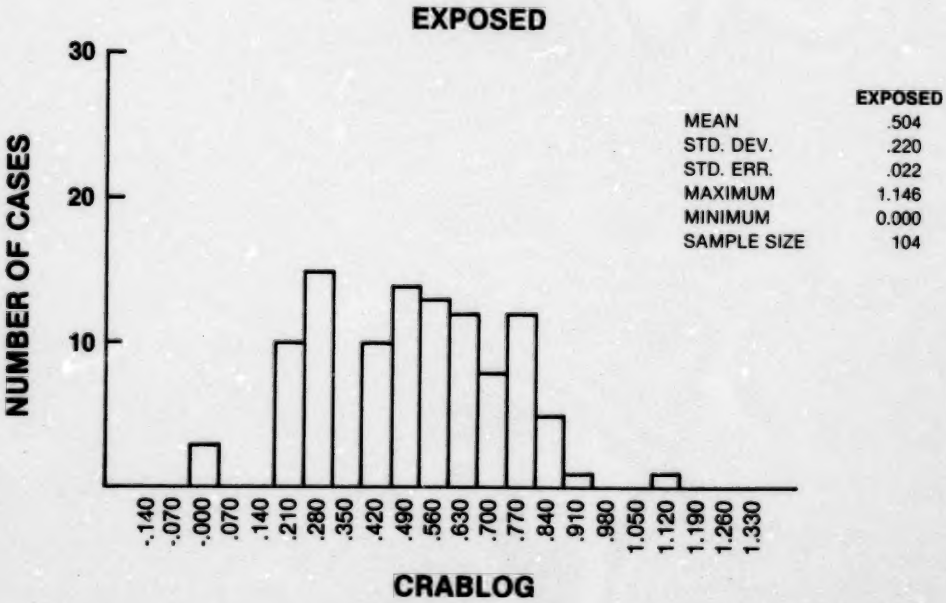
ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	TAIL PROBABILITY
BETWEEN GROUPS	.0024	1	.0024	5.92	.0159
WITHIN GROUPS	.0768	188	.0004		
TOTAL	.0792	189			

FIGURE 9.

A histogram showing CRABLOG (var. 5) for control and exposed groups





ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	TAIL PROBABILITY
BETWEEN GROUPS	.3270	1	.3270	6.97	.0090
WITHIN GROUPS	8.8236	188	.0469		
TOTAL	9.1505	189			

FIGURE 10.

A scattergraph showing SCEs and ARSENIC concentration. See figure 7 for explanation of numbers.

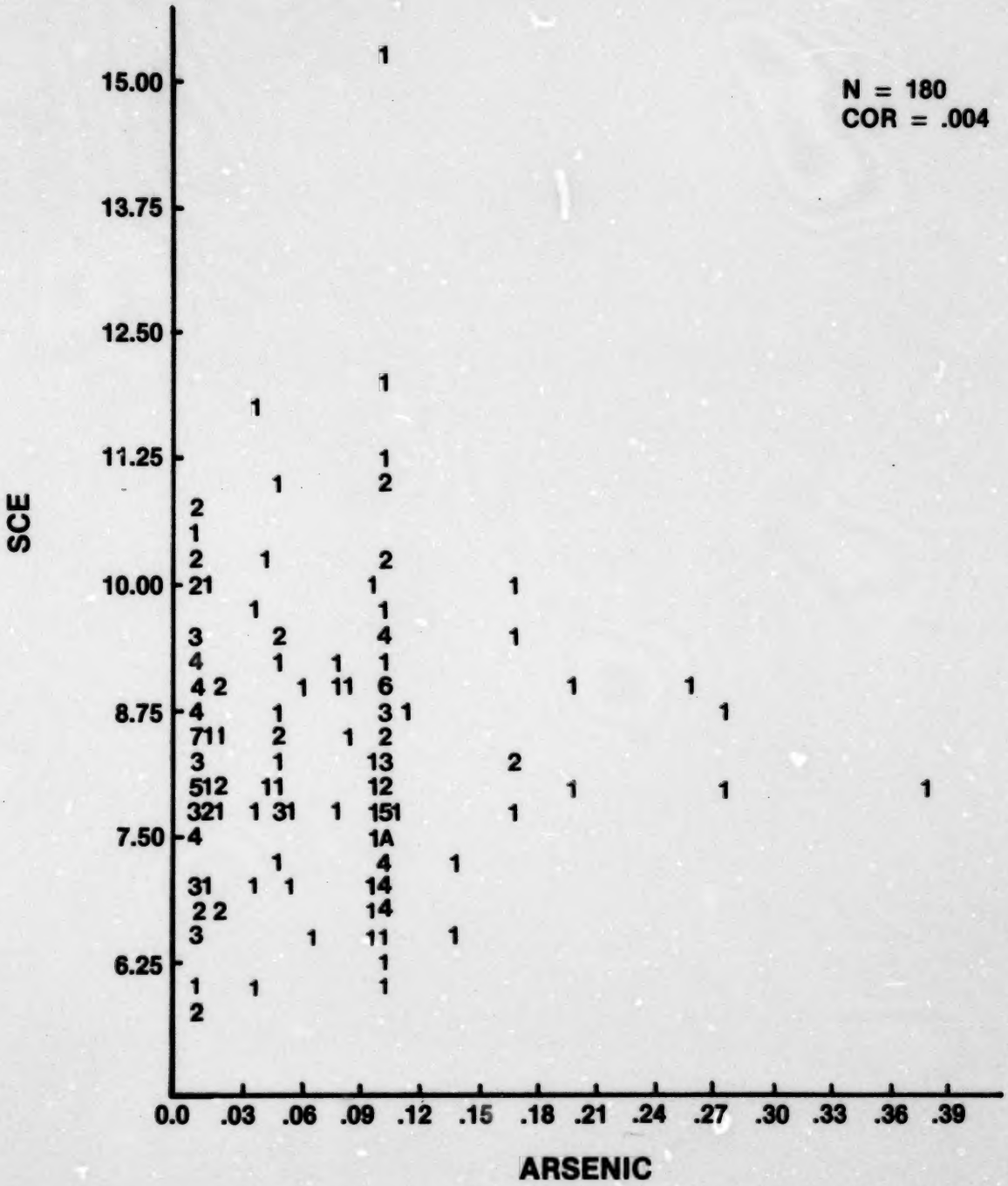
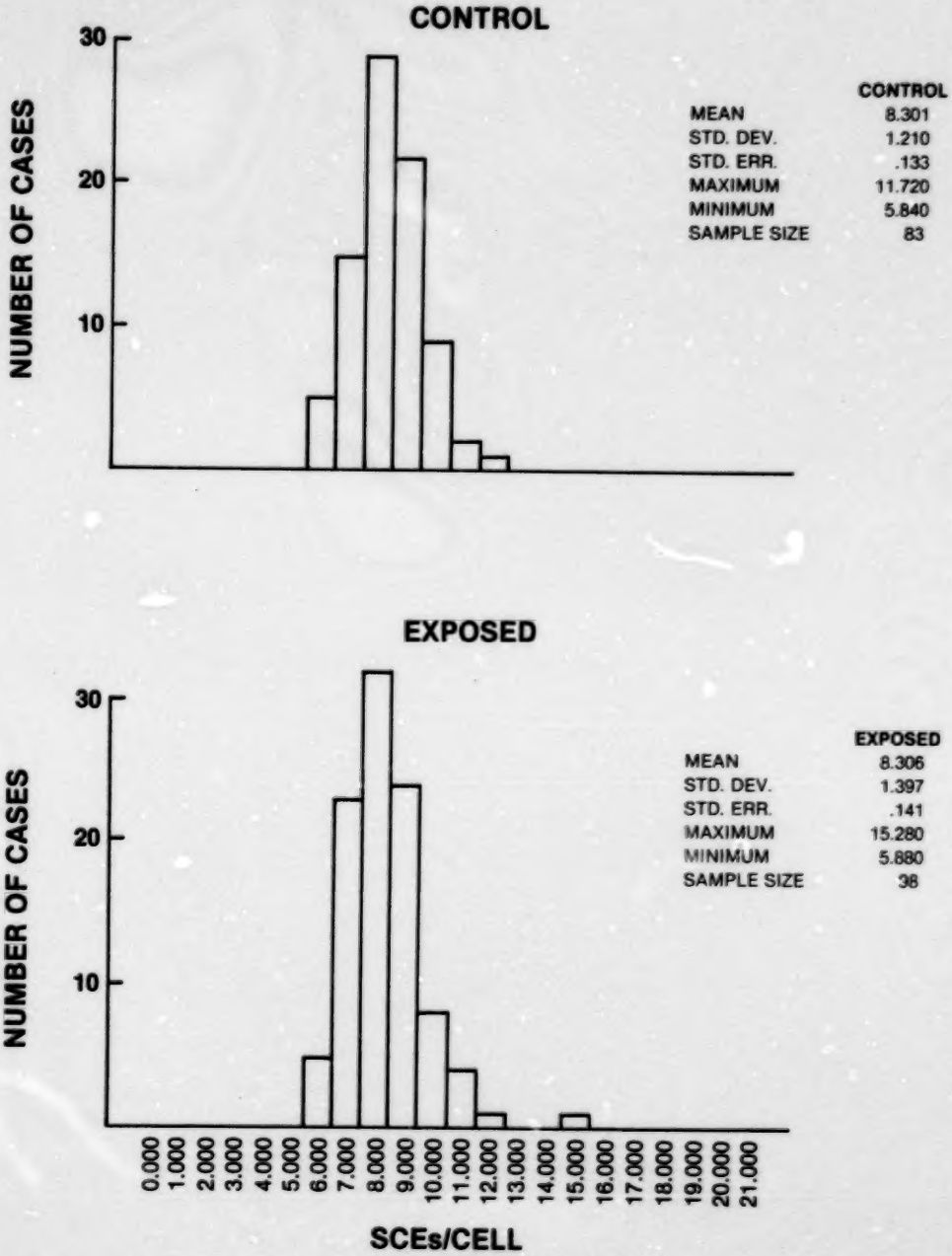


FIGURE 11.

Histogram of SCE (var. 6) for control and exposed groups.



ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	TAIL PROBABILITY
BETWEEN GROUPS	.0014	1	.0014	.00	.9772
WITHIN GROUPS	309.4016	179	1.7285		
TOTAL	309.4030	180			

FIGURE 12.

VICTOR J. LOCICERO, M.D.
MEDICAL DIRECTOR

INSTITUTE DIRECTOR

APPENDIX

Nevada Mental Health Institute

P.O. Box 2460 · TELEPHONE 322-6961
RENO, NEVADA 89505

INFORMED CONSENT FOR ARSENIC STUDY

I_____hereby give my consent to the staff of the Nevada Mental Health Institute for the collecting and use of my blood for their study dealing with cytological effects of arsenic in drinking water.

The nature and purpose of this study, the risks involved and any possible outcome of my participation in this study have been explained to me. I am also aware that all results stemming from this study, referring directly to me, will be held with confidentiality and shall be used only for statistical purposes.

I CERTIFY THAT I HAVE READ AND FULLY UNDERSTAND THE ABOVE CONSENT TO THE STUDY AND THAT THE EXPLANATIONS THEREIN REFERRED TO WERE MADE BEFORE I SIGNED.

_____ Date

_____ Signature of Participant

_____ Witness

When participant is a minor or incompetent to give consent:

_____ Date

_____ Signature of person authorized to consent for participant

_____ Witness

_____ Relationship to participant

_____ As. #

I.D. # A3-181

Name (optional) _____

Address (optional) _____

Telephone # (optional) _____

Interviewer _____

Date of Interview ___/___/___
mo. day yr.

Arsenic Content _____ ppm.

Analyzed by _____ On _____.

1. SEX Male _____, Female _____

2. RACE, ETHNICITY
   Caucasion _____
   Oriental _____
   Black _____
   Spanish _____
   Other _____

3. How old are you? _____ Years

4. What is your date of birth? ___/___/___
   mo. day yr.

5. What is/was your main occupation? And your place of work?

_____

6. Have you ever worked where you had contact with pesticides, herbicides or weed killers? (Do not include casual contact in your home garden).

Yes_____
No_____

If Yes:

A. What did you do in this job?

_____

B. Where was this? (Name of company, city and state)

_____

C. When did you first work with these chemicals? (Dates) ____/____
mo. yr.

D. When did you last work with them? (Dates) ____/____
mo. yr.

E. Did you work with these substances continously or only part of the time?

Continously_____
Part Time_____

F. Can you tell me the names of the substances you worked with?

_____

_____

7. Have you ever worked for a smelter that handled ores of copper, lead, zinc, gold, or silver?

Yes_____
No_____

If yes:

A. What is the name of the plant and its location (City and State?)

_____

B. What did you start to work at the smelter? (Dates) ____/____
mo. yr.

C. When did you last work there? (Dates) ____/____
mo. yr.

D. During that time, did you work there continously or only part of the time?

Continously_____
Part Time_____

8. Have you ever worked in a chemical paint?

Yes_____
No_____

If Yes:

A. What kind of chemicals were involved?

_____

_____

B. What is the name of the plant and location (City and State?)

_____

_____

C. Did the plant use or produce any substance containing arsenic?

Yes_____
No_____
Don't Know_____

D. Did the plant use or produce any substance containing selenium?

Yes_____
No_____
Don't Know_____

A. What substance was that?

_____

E. When did you first work there? (dates) _____/_____
mo. yr.

F. When did you last work there? (Dates) _____/_____
mo. yr.

G. During that time did you work there continously or only part of the time?

Continously_____
Part Time_____

9. Does your drinking water come from the public water company or your own private well?

Public_____
Well_____
Both_____
Other_____
Specify_____

A. For how many years has your water come from this supply? _____

B. Do you have a water softener?

Yes_____
No_____

C. Do you have a special filter on your water?

Yes_____
No_____

a. What is the name of the filter?_____

10. How many standard 8 oz glasses of this water do you drink in a 24 hour day in,

Summer_____, Winter_____.

11. How many cups of coffee, tea or other hot drinks do you have in a 24 hour day in,

Summer_____, Winter_____.

12. How many standard 8 oz. glasses of this water do you have in a 24 hour day made into drinks such as orange juice, Koolaide or ice tea in,

Summer_____, Winter_____.

13. Do you use <u>this water</u> in your home for cooking?

Yes_____
No_____

14. How many bottles or cans of soft drinks do you have in a 24 hour day in,

Summer_____,Winter_____.

15. How many glasses of wine do you drink in a 24 hour day in,

Summer_____ Winter_____.

16. Over the past year have you changed the <u>amount</u> of water you drink other than seasonal changes?

Yes_____
No_____

17. Do you smoke cigarettes?

Yes_____
No_____

A. How many packs a day?_____

18. Do you use Selsun or Selsun Blue Shampoo? Yes _____ No _____

19. Have you ever had appendicitis?

Yes_____
No_____

20. Have you ever been treated by a doctor for a parasitic disease such as tapeworm or roundworm (not pinworms)?

Yes_____
No_____

Card No. 4
I.D. #

21. Tell me if you have had any of the following health problems in the last two years

A. Liver disease? (hepatitis, yellow jaundice)

Yes_____
No_____

B. Kidney disease?

Yes_____
No_____

C. Diseases of the nerves such as paralysis or numbness?

Yes_____
No_____

D. Emotional or mental (nervous) illness?

Yes_____
No_____

E. Heart trouble?

Yes_____
No_____

F. Cancer? (especially of the skin)?

Yes_____
No_____

G. Arthritis?

Yes_____
No_____

H. Ulcers of stomach or intestines? Yes_____ No_____

I. Anemia or low blood?

Yes_____
No_____

J. A broken bone?

Yes_____
No_____

J. Which bone was that?_____When was that?____/____How long_____
mo. yr. mo.

22. Did you ever have any Miscarriage or problems with a new born baby?

Yes_____
No_____

If Yes, Explain_____

_____

23. Have you experienced any of the following during the past year?

		Yes	No
A. Tremors or shaking if the hands?	A.		
B. Unusual coldness of the fingers or toes, such as during warm weather?	B.		
C. More severe or more frequent headaches?	C.		
D. Decrease in touch or temperature sensations in your hands?	D.		
E. Decrease in touch or temperature sensations in your feet or legs?	E.		
F. A tingling of your naval (belly button)?	F.		
G. A loss of memory which interferes with your usual activities?	G.		
H. More frequent or longer lasting depression, or feeling blue?	H.		
I. Any unusual weight loss?	I.		
J. A loss of appetite?	J.		
K. Trouble with your energy level that has made your daily activities harder?	K.		
L. Trouble with a stiff neck?	L.		
M. Feelings of dizziness or trouble with your balance?	M.		
N. Trouble with loose stools or diarrhea?	N.		

		Yes	No
O. Trouble with nausea or vomiting?	O.		
P. Trouble with stomach pains or cramps?	P.		
Q. Darkening of any skin areas?	Q.		
R. Any unusual or unexplained skin rashes? or excessive itching?	R.		
S. Trouble with your nails becoming brittle?	S.		
T. More trouble than usual with dandruff?	T.		
U. Any abnormal loss of hair?	U.		
V. Any unusual loss of nails?	V.		
W. Any unusual pain in muscles or joints?	W.		
X. Any swelling of your ankles?	X.		

For Adult Females Only

24. Any irregular menstrual periods?

Yes_____
No_____
Not Applicable_____

25. Do you eat any of these foods at least once a week, average:

	No (1)	1 x/wk (2)	2-3 x/day (3)	1 x/day (4)	Several x /day (5)	In Past 2 Days Yes (1)	No (2)
Fish or shellfish							
Kidney or Kidney dishes							
Liver or liver dishes							
eggs-alone or in dishes							
Bread or baked goods							

26. Do you eat locally grown vegetables (raise your own or buy from a local grower)?

Yes_____
No_____

27. Do you can or freeze these vegetables for winter use?

Yes_____
No_____

28. Do you eat local poultry or livestock (raise or buy from local rancher)?

29. What was your highest grade you completed in school?

	00 01 02 03
	04 05 06 07
	08 09 10 11
	12
College/other post high school	13 14 15 16
Graduate school/other post college	17 18 19 20+

30. (optional) Which of these categories best describes your total family income before taxes for the past year? (Please consider all sources) Just give me the category.

A. LESS THAN $5,000
B. $5,000 - $9,999
C. $10,000 - $14,999
D. $15,000 - $19,999
E. $20,000 - $29,999
F. MORE THAN $30,000

That is the end of your questionnaire. Thank you very much for your help.

FORM A

I.D. # — — — — —

Disease & Question No.	A Did the doctor have a specific name for this illness?	B When did this start?		C Do you still have this problem?		D When did you get over this problem?		E When was the first time you ever had this problem?	
		mo.	yr.	yes	no	mo.	yr.		
					ASK D				

FORM B

I.D. # ___ ___ ___ ___ ___

Symptom & Question No.	A When did you first notice this problem?	B Do you still have this problem?		C How long did you have this problem?	D Did you see a doctor about this problem?		E What did he say the problem was?	F When was the first time you ever noticed this problem?	
		Yes	No		Yes	No			
		ASK D	ASK C		ASK E				

I.D. #_____

Form C
Pregnancy Complications And Miscarriages

Approx Date	What complications did you have if any.	Was the duration		If miscarriage(s) occured			Any birth defect amoung your children		Any such problems amoung your relatives	
		Normal	Premature	Pregnacy duration	Reason	Other Information	Age	Sex		